TOTAL SYNTHESES OF THE TAMBJAMINE ALKALOIDS AND CERTAIN EPOXYQUINOL-TYPE NATURAL PRODUCTS

A Thesis submitted for the degree of

Doctor of Philosophy of the Australian National University

By

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August 2010

DECLARATION

I declare that, to the best of my knowledge, the material presented in this thesis represents the result of original work carried out by the author during the period 2006-2010 and has not been presented for examination for any other degree. This thesis is less than 100,000 words in length. Established methodologies have been acknowledged, wherever possible, by citation of the original publications from which they derive.

David Pinkerton

August 2010

ACKNOWLEDGEMENTS

I am sincerely grateful to my supervisor, Professor Martin Banwell, for his considerate support and astute guidance. Professor Banwell's encouraging attitude has been invaluable and I am grateful too for his support of my attendance at conferences in Australia and abroad.

The members of the Banwell group have proven themselves to be not only a studious cohort but very friendly as well. The pleasing social and stimulating scientific environment engendered by the character of my colleagues has bolstered my enthusiasm for chemical research. I would like to acknowledge the positive contribution that such high-calibre colleagues have made to my period of research at the ANU. Doctors Xing Ma and Gwion Harfoot kindly shared their laboratory space with me during my induction into the group and assisted me in honing certain techniques of chemical separation and the conduct of reactions under inert atmospheres. More recently, I have had the pleasure of jointly publishing work with two fellow group members, Mister Lorenzo White and Doctor Christine Dietinger, which culminated in the synthesis of the epoxyquinol natural product (+) isoepiepoformin.

I gratefully acknowledge useful discussions on the biological properties of the tambjamine alkaloids with Doctor Cláudia Pessoa, and Professors Mary Garson and Naresh Kumar. It was exciting to be involved in an international collaborative effort to study these intriguing molecules and then see this work accepted for publication.

Doctor Anthony Willis carried out all of the single-crystal X-ray analyses that are depicted in this thesis. These analyses have proven immensely valuable for the success of my work. Likewise, I am especially indebted to assistance rendered readily by the microanalytical services unit and the mass spectrometry unit at the RSC. Administrative staff at the RSC have been exceptionally helpful and this is appreciated.

I acknowledge the receipt of an ANU PhD Scholarship, and Alan Sargeson PhD and RSC supplementary scholarships.

My thanks also extend to my kind volunteer proof-readers, Messrs Phillip Gatt and Pat Sharp and Doctor Jennifer Hodgson. I am especially appreciative of Doctor Hodgson's companionship and continuous support during my degree.

PUBLICATIONS AND PRESENTATIONS

The following list details the publications and presentations that have resulted from research performed during the period of candidature for the degree of Doctor of Philosophy.

Publications:*

White, L.; Dietinger, C. E.; Pinkerton, D. M.; Willis, A. C., Banwell, M. G.; An enantioselective synthesis of the epoxyquinol (+)-isoepiepoformin. *European Journal of Organic Chemistry*, in press.

Pinkerton, D. M.; Banwell, M. G.; Garson, M. J.; Kumar, N.; de Moraes, M. O.; Cavalcanti, B. C.; Barros, F. W. A.; Pessoa, C., Biological evaluation of synthetically-derived tambjamines C, E, F, G, H, I and J, BE-18591 and a related alkaloid from the marine bacterium *Pseudoalteromonas tunicata*. *Chemistry and Biodiversity* **2010**, *7*, 1311-1324.

Pinkerton, D. M.; Banwell, M. G.; Willis, A. C., (+)-Hexacyclinol. *Acta Crystallographica Section E: Structure Reports Online* **2010**, *66*, O342-O343.

Pinkerton, D. M.; Banwell, M. G.; Willis, A. C., Chemoenzymatic syntheses of the epoxyquinols (−)-bromoxone acetate and (−)-tricholomenyn. *Australian Journal of Chemistry* **2009**, *62*, 1639-1645.

Pinkerton, D. M.; Banwell, M. G.; Willis, A. C., Chemoenzymatic access to versatile epoxyquinol synthons. *Organic Letters* **2009**, *11*, 4290-4293.

Pinkerton, D. M.; Banwell, M. G.; Willis, A. C., Total syntheses of tambjamines C, E, F, G, H, I and J, BE-18591, and a related alkaloid from the marine bacterium *Pseudoalteromonas tunicata*. *Organic Letters* **2007**, *9*, 5127-5130.

* A copy of each of these publications is provided as a PDF on the compact disc found on the inside back cover of this thesis.

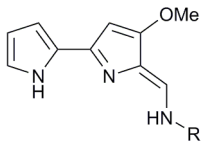
Presentations:

Pinkerton, D. M.; Banwell, M. G.; Willis, A. C., Total syntheses of tambjamines C, E, F, G, H, I and J, BE-18591, and a related alkaloid from the marine bacterium *Pseudoalteromonas tunicata*. Poster presentation at the 11th Belgian Organic Synthesis Symposium, Ghent, Belgium, 13-18 July 2008.

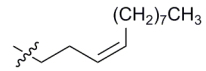
Pinkerton, D. M.; Banwell, M. G.; Willis, A. C., Towards the synthesis of the tambjamine alkaloids. Poster presentation at the 22nd Royal Australian Chemical Institute Organic and Physical Chemistry Conference, Adelaide, Australia, 28 January – 2 February 2007.

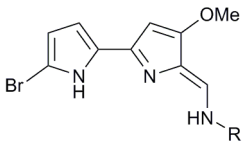
ABSTRACT

The tambjamines (**1**-**12**) are a subset of the 4-methoxybipyrrolic class of natural products. They are structurally related to the tripyrrolic prodigiosins and, like these compounds, show some intriguing biological effects such as the inhibition of immunoproliferation in rabbits (for example tambjamine **5** aka BE-18591). They also bind to duplex DNA and effect cleavage of it in the presence of copper (II) without the aid of an external reductant. Despite exhibiting these interesting properties, no total synthesis of the tambjamines had been previously described and neither had any comprehensive biological assessment of these compounds been published. Accordingly, Chapter One of this thesis reports on the total synthesis of tambjamines **2**-**6** and **8**-**11** and the outcomes of their evaluation as anti-microbial and cytotoxic agents.

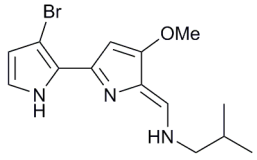


1 R=H (T.A)
2 R=CH_2CH_3 (T.E)
3 R=$(CH_2)_2Ph$ (T.F)
4 R=$CH_2CH(CH_3)_2$ (T.C)
5 R=$(CH_2)_7CH_3$ (BE-18591)
6 R=



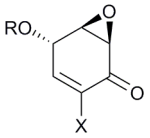


7 R=H (T.B)
8 R=CH_2CH_3 (T.G)
9 R=$(CH_2)_2CH_3$ (T.H)
10 R=$(CH_2)CH(CH_3)_2$ (T.I)
11 R=$CH_2CH(CH_3)CH_2CH_3$ (T.J)

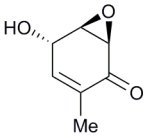


12 (T.D)

Chapter Two introduces a different, but large and growing class of epoxyquinol-type natural products, members of which display a wide range of biological effects. Representative epoxyquinol-type natural products include (+)-bromoxone (**55**), (+)-bromoxone acetate (**106**), (+)-epiepoformin (**108**), (−)-harveynone (**148**) and (−)-tricholomenyn A (**174**).



55 R = H
106 R = Ac



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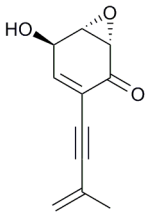

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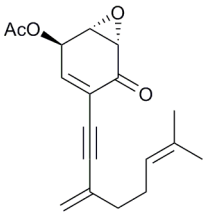

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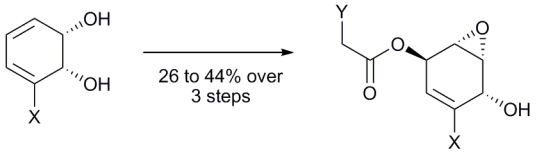
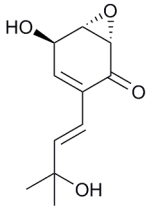
In an effort to address the challenges associated with producing epoxyquinol natural products on scales useful for the establishment of detailed structure-activity relationships (SAR), selective methods for the elaboration of the versatile epoxyquinol synthons **98**-**100**, **107** and **188** from enantiomerically pure *cis*-dihydrocatechols **88**-**90** were devised. The former group of compounds were then converted, generally by straightforward means, into the natural products *ent*-**55**, *ent*-**106**, *ent*-**108**, **148** and **174**.



88 X = Cl
89 X = Br
90 X = I

98 X = Cl; Y = Cl
99 X = Br; Y = Cl
100 X = I; Y = Cl
107 X = Br; Y = H
188 X = I; Y = H

By related means compounds **199** and **244** were prepared and then shown to engage in Diels-Alder dimerization reactions to give the dimeric epoxyquinol natural products (+)-panepophenanthrin (**192**) and (+)-hexacyclinol (**233**), respectively.



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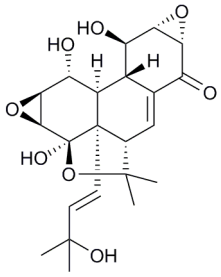

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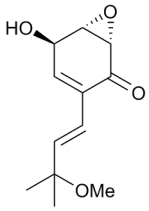

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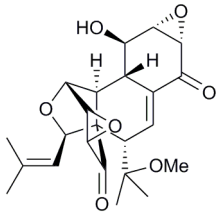

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GLOSSARY

The following abbreviations have been used throughout this thesis:

δ	chemical shift (parts per million)
°C	degrees Celsius
µg	microgram(s)
µL	microlitre(s)
Ac	acetyl
AcOH	acetic acid
aq.	aqueous
Ar	(unspecified) aryl group
atm	atmosphere(s)
Bn	benzyl
br	broad
Bu	butyl
n-Bu	*n*-butyl
s-Bu	*sec*-butyl
t-Bu	*tert*-butyl
c	concentration (g/100 mL)
ca.	*circa* (approximately)
cat.	catalytic/catalyst
cf.	*confer* (compare)
cm	centimeter(s)
conc.	concentrated
m-CPBA	*meta*-chloroperbenzoic acid
CSA	(1*S*)-(+)-10-camphor sulfonic acid
d	doublet
DA	(intermolecular) Diels-Alder
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	diethyl azodicarboxylate
c-DHC	*cis*-1,2-dihydrocatechol
DIBAL-H	di*iso*butylaluminium hydride
DMAP	4-(*N*,*N*-dimethylamino)pyridine
DME	1,2-dimethoxyethane
DMF	*N*,*N*-dimethylformamide

DMP	Dess-Martin periodinane
2,2-DMP	2,2-dimethoxypropane
DMSO	dimethyl sulfoxide
ee	enantiomeric excess
e.g.	*exempli gratia* (for example)
EI	electron impact
equiv.	equivalents
ES	electrospray (mass spectrometry)
Et	ethyl
et al.	*et alia* (and others)
Et_3N	triethylamine
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
eV	electron volt(s)
FGI	functional group interconversion
g	gram
GC	gas chromatography
gem	geminal
gen.	generation
h	hour(s)
HRMS	high resolution mass spectrometry
hν	light
Hz	Hertz
IMDA	intramolecular Diels-Alder
IBX	2-iodoxybenzoic acid
IR	infra-red
J	^{1}H-^{1}H coupling constant (in Hz)
kbar	kilobar(s)
KHMDS	potassium bis(trimethylsilyl)amide
L	litre(s)
LDA	lithium di*iso*propylamide
LiHMDS	lithium hexamethyldisilazide
lit.	literature value
LiTMP	lithium 2,2,6,6-tetramethylpiperidine
LRMS	low resolution mass spectrometry

m	multiplet
M	molar
$M^{+\bullet}$	molecular ion
Me	methyl
MeOH	methanol
MHz	mega-Hertz
min	minute(s)
mL	millilitre(s)
mm	millimetre(s)
mmol	millimole(s)
mol	mole(s)
m.p.	melting point (°C)
MS	mass spectrometry
m/*z*	mass-to-charge ratio
NaHMDS	sodium hexamethyldisilazide
NBS	*N*-bromosuccinimide
nm	nanometre(s)
NMR	nuclear magnetic resonance
ORTEP	Oak Ridge Thermal Ellipsoid Plot
P	unspecified protecting group
p	*para*
PCC	pyridinium chlorochromate
PDC	pyridinium dichromate
Ph	phenyl
PMB	*para*-methoxybenzyl
PPL	porcine pancreatic lipase
ppm	parts per million
PPTS	pyridinium *para*-toluenesulfonate
q	quartet
qu	quintet
quant.	quantitative
ref.	reference
R	(unspecified) alkyl group
R_f	retardation factor (as encountered in TLC)
r.t.	room temperature
s	singlet

t	triplet
t	tertiary
TASF	tris(dimethylamino)sulfonium difluorotrimethylsilicate
TBAF	tetra-*n*-butylammonium fluoride
TBS	*tert*-butyldimethylsilyl
temp.	temperature (°C)
TEMPO	2,2,6,6-tetramethyl-piperidine *N*-oxide
TES	triethylsilyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	tri*iso*propylsilyl
TLC	thin layer chromatography
TMEDA	*N*,*N*,*N*′,*N*′-tetramethylenediamine
TMS	trimethylsilyl
TPAP	tetrapropylammonium perruthenate
TsOH	*para*-toluenesulfonic acid
UV	ultra violet (spectroscopy)
viz.	*videlicit* (that is, namely)
vs.	*versus*
v/*v*	unit volume per unit volume (ratio)
W	watt(s)
w/*v*	unit weight per unit volume (%)

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CHAPTER ONE

THE TOTAL SYNTHESIS AND BIOLOGICAL EVALUATION OF THE TAMBJAMINES

1.1 INTRODUCTION

1.1.1 Isolation and characterisation of the tambjamines

The tambjamines (**1-12**, see Figure 1-1),[†] are a class of some dozen alkaloids that have been isolated from various marine sources including bryozoans, nudibranchs and ascidians.[1-6] In addition, the related alkaloids BE-18591 (**5**)[1] and **6**[2] have been obtained from cultures of *Streptomyces sp*. BA18591 and the marine bacterium *Pseudoalteromonas tunicata*, respectively. They all incorporate a (1*Z*)-1-[3-methoxy-5-(1*H*-pyrrol-2'-yl)-2*H*-pyrrolylidene]-methanamine core and are, therefore, structurally related to various polypyrrolic natural products including the prodigiosins (some of which are potent immunosuppressive and/or anti-cancer agents),[3,4] tambjamine tetrapyrrole (an inhibitor of cancer cell proliferation)[3,4] and streptorubin B (an immunomodulator).[5] A discussion of such related natural products follows in Section 1.1.2.

[†] For convenience, and because they each possess the same bipyrrolic core, all of the natural products **1-12** have been designated as tambjamines even though one of these, **6**, has been assigned the code-name BE-18591 while another, **12**, has only been identified as a "...new member of the tambjamine class..." and remains to be assigned a letter.

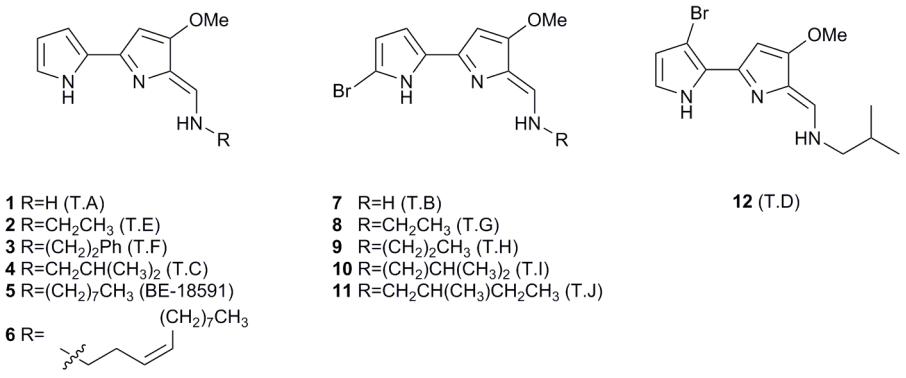


Figure 1-1: *Tambjamines A - J, BE-18591 and a related alkaloid from the marine bacterium* Pseudoalteromonas tunicata.

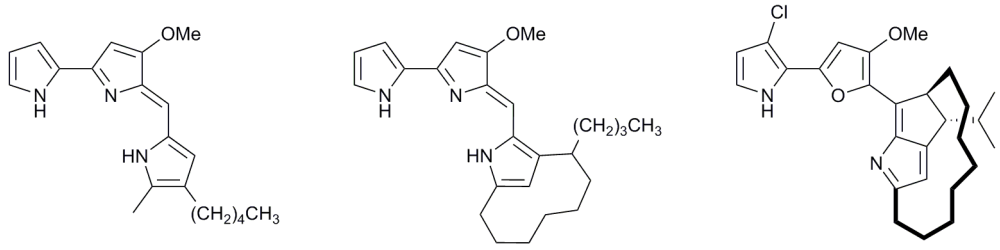
In some instances the tambjamines have been implicated in the chemical defense mechanisms of the organisms from which they were first obtained. A number of them have been shown to possess intriguing biological properties although the studies revealing these tend to have been conducted in conjunction with their isolation and, therefore, carried out in a piecemeal fashion. Thus, tambjamines A–D have been shown to act against *Escherichia coli*, *Staphylococcus aureus* and *Vibrio anguillarum*[6] while the last of these compounds displayed cytotoxic activity against several tumor cell lines, antimitotic effects in a sea urchin egg development assay, antifungal effects against *Candida albicans* and antibacterial activity against *Bacillus subtilis*.[7] Tambjamine D (the 3'-bromo-analogue of **10**) also shows concentration-dependent cytotoxic and genotoxic effects in cultured Chinese hamster lung fibroblasts (V79 cells)[8] while screening of tambjamine I (**10**) against a 60-cell line panel revealed that it had average GI_{50} and LD_{50} values of 1.6 and 18 μM, respectively.[9] BE-18591 (**5**) has been shown to inhibit immunoproliferation and gastritis in rabbits.[10] Certain tambjamines have also been observed to bind duplex DNA and can cleave this biomolecule in the presence of Cu(II). It is this last property that may be responsible for many of the reported biological effects of the tambjamines.[9-14] While the tambjamines have been manipulated chemically for the purposes of securing a library of analogues,[15] they do not appear to have been the subject of any total synthesis studies. Accordingly, the development of total syntheses of these intriguing alkaloids was considered a priority. Successful total syntheses would assist in confirming the structural assignment of these alkaloids. Furthermore, it was hoped that the production of useful quantities of the natural products would allow for a more comprehensive evaluation of their biological properties.

1.1.2 Other pyrrolic natural products closely related to the tambjamines

The tambjamines and related alkaloids are members of the 4-methoxybipyrrolic subdivision of pyrrole-containing natural products as they incorporate the 4-methoxy-2,2′-bipyrrole chromophore. These compounds are structurally related to the more well-known prodigiosins,

certain members of which have been the subject of total syntheses.[16-21] The tripyrrolic prodigiosins, such as compounds **7** and **8**, often exhibit strong coloration and it is believed that this property, and their presence in bacterial colonies, has contributed to some events being considered miraculous (or "prodigious").[22]

Roseophilin (**9**) is structurally reminiscent of the prodigiosins and the tambjamines but with the 4-methoxybipyrrolic unit having been exchanged for a 4-methoxyfuran moiety. Roseophilin also incorporates a chiral, *ansa*-bridged azafulvene core.



7 **8** **9**

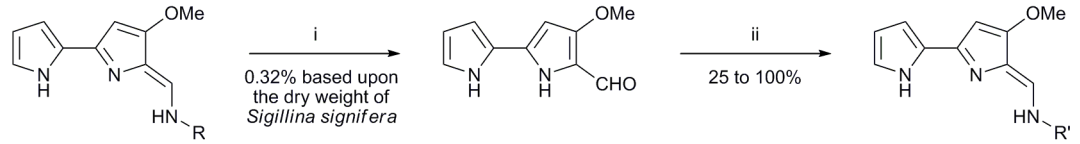
Figure 1-2: *Prodigiosin (left), the parent member of its class is shown alongside the closely related alkaloids streptorubin B (centre) and roseophilin (right).*

1.1.3 Previous studies on the synthesis of compounds related to the tambjamines

No total syntheses of the tambjamines had been described prior to the studies described herein. However, a consideration of the syntheses of the following related compounds is instructive.

Quinn's syntheses of a library of tambjamine analogues (2001)

Quinn *et al*. extracted a mixture of tambjamines C (**4**), E (**2**) and F (**3**) from Australian marine ascidian, *Sigillina signifera*, and hydrolysed this to afford 202 mg of aldehyde **17**.[15] Exposure of this aldehyde to a range of primary amines then afforded, *via* a Schiff-base condensation reaction, a combinatorial library of ten tambjamine analogues (Scheme 1-1). This work emphasized the utility of aldehyde **17** as a precursor to the tambjamines themselves as well as various analogues. The outcomes of the screening of this novel tambjamine library have not been published to date.



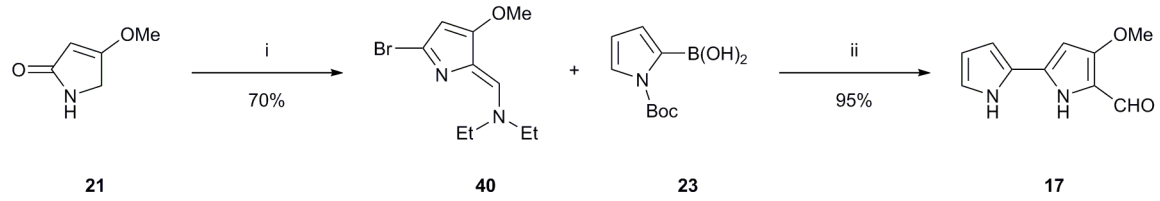
2 R=CH_2CH_3 (T.E)
3 R=$(CH_2)_2Ph$ (T.F)
4 R=$CH_2CH(CH_3)_2$ (T.C)

17

Scheme 1-1: *Reagents* (i) KOH, MeOH; (ii) one of sixteen primary amines, acetic acid.

Lavallée's two-step synthesis of 4-methoxy-2,2'-bipyrrole-5-carboxaldehyde (2006)

Protracted and low-yielding syntheses of aldehyde **17**[20,23,24] have been such that, as is described above, Quinn *et al*. opted to obtain this pivotal compound through the hydrolysis of tambjamines obtained from natural sources.[15] However, in 2006 Lavallée and co-workers described a two-step process for the conversion, in 67% overall yield, of the commercially-available lactam **21** into aldehyde **17**.[25] In order to accomplish this transformation, the starting material **21** was reacted with a Vilsmeier complex obtained from the combination of phosphoryl tribromide and diethyl formamide. As a result, azafulvene **40** was obtained. Suzuki cross-coupling of this material with the previously described pyrrole boronic acid **23** proceeded in a high yield. Under these conditions, the *tert*-butoxycarbonyl protecting group was cleaved and the enamine residue was hydrolyzed to the desired aldehyde, processes that were likely to have occurred during the work-up process.



21 **40** **23** **17**

Scheme 1-2: *Reagents* (i) $POBr_3$, $Et_2NC(O)H$; (ii) $Pd(PPh_3)_4$, Na_2CO_3.

This sequence could be performed on a multi-kilogram scale and product **17** has been used to prepare compound GX15-070 (**41**) in sufficient quantities for comprehensive testing of it as a potential oncolytic agent.[26]

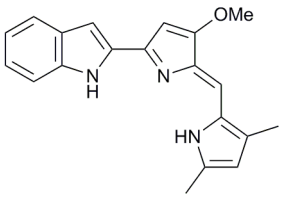


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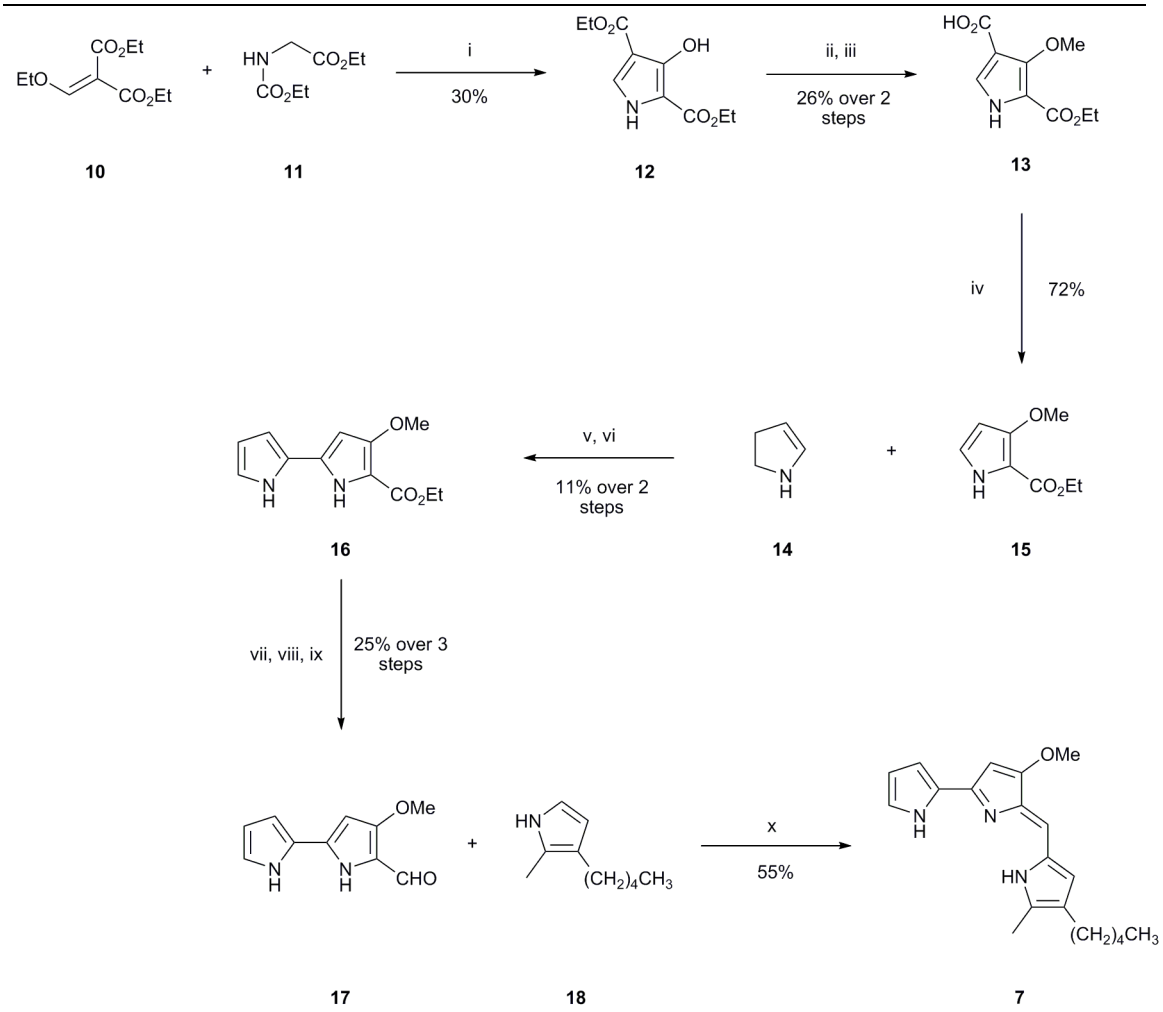
Figure 1-3: *GX15-070 (41)*

Rapoport and Holden's total synthesis of prodigiosin (1962)

The total synthesis of prodigiosin carried out by Rapoport and Holden in the early sixties hinged on the generation of the pivotal intermediate 4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (**17**).[20] The means by which this aldehyde was obtained have been exploited in a number of subsequent syntheses. The process devised by Rapoport and Holden is shown in Scheme 1-3 and involves the application of the Knorr pyrrole synthesis to the known compounds, diethyl ethoxymethlenemalonate and ethyl *N*-ethoxycarbonylglycinate. Methylation of the ensuing alcohol, **12**, followed by selective and acid-catalyzed ester hydrolysis then afforded the

thermally-sensitive pyrrole **13**. Heat-induced proto-decarboxylation provided pyrrole **15** and this compound was sufficiently reactive to engage the Schiff base obtained from 2-pyrroline under acidic conditions to afford a bicyclic enamine intermediate. Catalytic dehydrogenation of this enamine was used to aromatize the left-hand azacycle and thereby delivered the requisite bipyrrole (**16**). The McFadyen-Stevens reaction was then used to reduce the ethyl ester to the desired aldehyde (**17**). This last step involved the formation of the corresponding acylsulfonylhydrazide from ester **16** and then decomposition of it with base. In the closing stages of the total synthesis, aldehyde **17** was condensed with 2-methyl-3-amylpyrrole (**18**) in the presence of a catalytic quantity of acid. In this way, the first total synthesis of prodigiosin (**7**) was achieved.

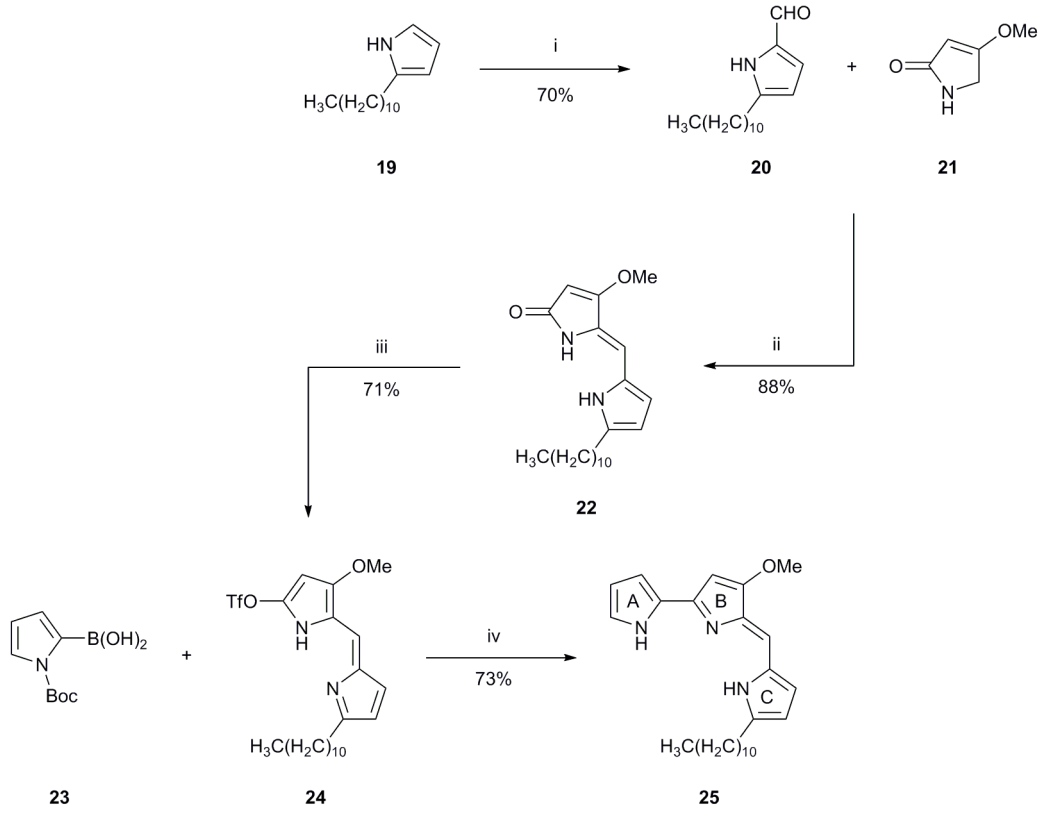


Scheme 1-3: *Reagents* (i) Na; (ii) CH_2N_2; (iii) sulfuric acid, H_2O; (iv) 300 °C in a sublimation apparatus; (v) 150 °C in a sealed tube; (vi) Pd/C, *p*-cymene; (vii) hydrazine; (viii) *p*-toluenesulfonyl chloride, pyridine; (ix) Na_2CO_3; (x) HCl.

D'Alessio and Rossi's total synthesis of undecylprodigiosin (1996)

The emergence of transition metal cross-coupling reactions has led to a new approach to the synthesis of the pyrrole-based alkaloids. Unlike earlier syntheses that relied upon the

preparation of an intermediate 2,2′-bipyrrole moiety, D'Alessio and Rossi postponed the formation of this motif until the last step of the four-step reaction sequence that afforded undecylprodigiosin (**25**) (Scheme 1-4).[16] Thus, the known 2-undecylpyrrole **19** underwent regioselective formylation under Vilsmeier-Haack conditions to afford aldehyde **20**. In the presence of hydroxide, compound **20** and the previously described α,β-unsaturated γ-lactam **21** engaged in an aldol-type condensation reaction that afforded the pyrrolomethylene derivative **22**. Exposure of this last compound to trifluoromethanesulfonic anhydride delivered triflate **24** that engaged the known and readily available boronic acid **23** in a Pd[0]-catalyzed Suzuki Miyaura cross-coupling reaction to give target **25**. The utility of late-stage coupling of the A-ring was demonstrated recently in preparing a collection of C-ring functionalized prodigiosin analogues.[21]



Scheme 1-4: *Reagents* (i) DMF, $POCl_3$; (ii) NaOH; (iii) Tf_2O; (iv) Pd[0], K_2CO_3.

Fürstner's total synthesis of nonylprodigiosin (1999)

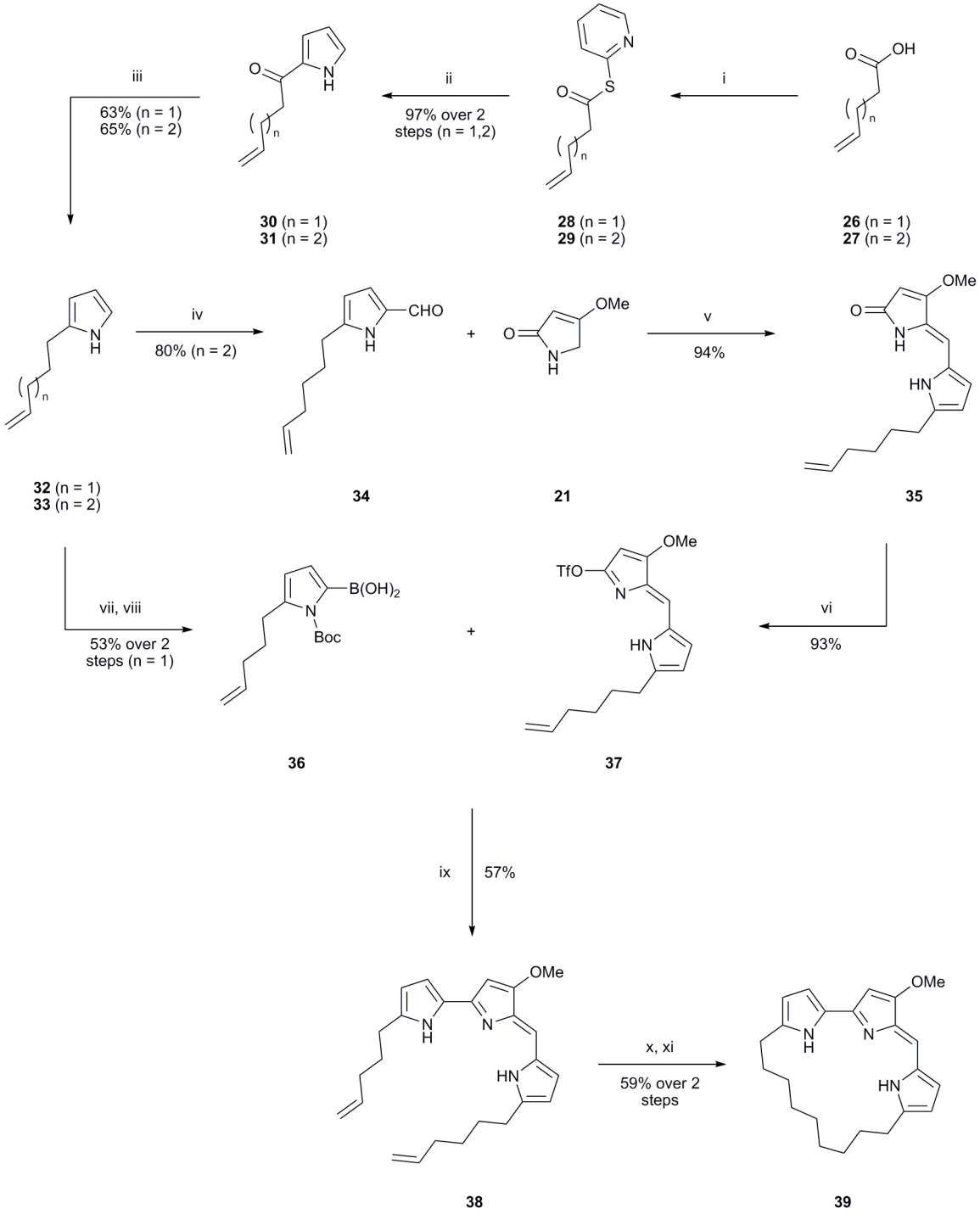
Having described the first total synthesis of roseophilin (**9**) in 1997,[27] Fürstner *et al*. proceeded to develop the first total synthesis of nonylprodigiosin.[17] The sequence incorporated, as a key step, a Pd[0]-catalyzed cross coupling reaction reminiscent of that developed by D'Allesio and Rossi.[16] A ring-closing metathesis reaction was employed in the penultimate step of this synthesis in order to establish the requisite nineteen-membered macrocycle. Thus, pyrroles **32** and **33**, differing only in the length of their olefinic side-chains, were prepared in parallel sequences from unsaturated carboxylic acids **26** and **27**, respectively (Scheme 1-5). The

preparation of these intermediates proceeded *via* the corresponding 2-pyridylthioesters **28** and **29**, respectively. These compounds readily *C*-acylate pyrrole thus affording ketones **30** and **31**. Presumably the electron-rich nature of the pyrrole ring facilities the complete reduction of the carbonyl functional group in the latter compounds and thereby allows their ready conversion into the somewhat-unstable pyrroles **32** and **33**, respectively.

Of pyrroles **32** and **33**, the one bearing the shorter side-chain (**32**) was selected for conversion into the boronic acid coupling partner. Under conditions described below, the other pyrrole, **33**, was converted into the triflate **37** required in the projected palladium-catalyzed cross-coupling reaction. Vilsmeier-Haack formylation of pyrrole **33** afforded aldehyde **34** that engaged in a dehydrative coupling with lactam **21**. Compound **35** so formed was treated with triflic anhydride to give triflate **37**.

The desired boronic acid coupling partner (**36**) was obtained in two steps from pyrrole **32**. In order to avoid unwanted reaction at nitrogen, as well as to aid in directing subsequent metallation, this position was protected with the *tert*-butoxycarbonyl group. As a result, deprotonation took place at the 5-position and the ensuing metallated intermediate was quenched with trimethyl borate. Addition of dilute hydrochloric acid facilitated the hydrolysis of the resulting boronic ester and thereby furnished the other coupling partner, namely acid **36**.

The boronic acid **36** and triflate **37** were united using Suzuki cross-coupling chemistry and in a manner analogous to that applied by D'Alessio and Rossi *en route* to alkaloid **25**. In this instance, the reaction proceeded in a moderate yield and delivered RCM-precursor **38**. A modified ruthenium catalyst derived from *bis*(triphenylphosphine)ruthenium(II) chloride effected the pivotal ring-closing metathesis reaction that produced the anticipated *trans*-olefinic macrocycle. The isolated alkene, present in this intermediate, was removed by hydrogenation in the presence of a ruthenium (I) catalyst. As a result, nonyl prodigiosin (**39**) was obtained in 59% combined yield over the final two steps. Fürstner *et al.* later carried out the total synthesis of butylcycloheptylprodigiosin, a related alkaloid, using very similar chemistry.[19]



Scheme 1-5: *Reagents* (i) Bis(2-pyridyl) disulfide, PPh_3; (ii) pyrrolylmagnesium chloride; (iii) $NaBH_4$, *i*-PrOH; (iv) $POCl_3$, DMF; (v) NaOH; (vi) triflic anhydride; (vii) Boc_2O, DMAP; (viii) lithium 2,2,6,6-tetramethylpiperidinide; $B(OMe)_3$; $HCl_{(aq.)}$; (ix) $Pd(PPh_3)_4$, $Na_2CO_{3\,(aq.)}$ LiCl; (x) indenylidene ruthenium complex[17]; (xi) $RhCl(PPh_3)_3$, H_2.

1.2 SYNTHETIC STRATEGY ASSOCIATED WITH THE PRESENT SYNTHESIS OF THE TAMBJAMINES

While naturally-derived samples of the tambjamines have been manipulated chemically for the purposes of securing a library of analogues,[15] they have not been subjected to any total synthesis

studies prior to the work described here.[28] As noted earlier, to the best of our knowledge the outcomes of the screening of the above-mentioned library have not been published.

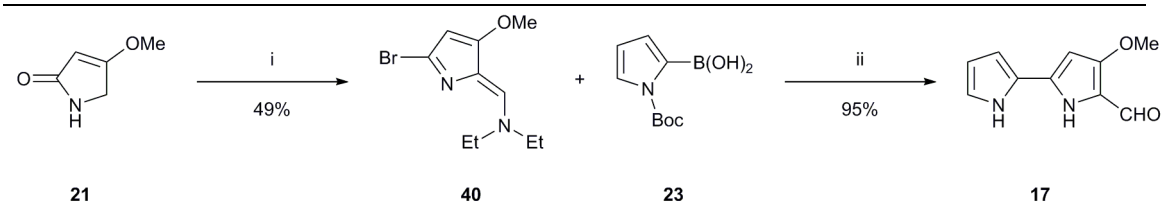
The recently reported and very concise synthesis of aldehyde **17** by Lavallée and co-workers provided ready access to a pivotal intermediate associated with various syntheses of prodigiosin and its analogues.[3,20,23,29-33] It was anticipated that this same compound would also serve to underpin the work reported in the following section and allow for the completion of the first total syntheses of the tambjamines.

1.3 TOTAL SYNTHESES OF CERTAIN NON-BROMINATED TAMBJAMINES

The non-brominated tambjamines (**1-6**) were selected as the first targets of total synthesis as it was considered that these compounds would be the easiest to obtain. As well, this endeavour would present an opportunity to develop methodology that would later be useful in the synthesis of brominated congeners **7-12**.

1.3.1 The synthesis of 4-methoxy-2,2'-bipyrrole-5-carboxaldehyde

As reported by Lavallée and co-workers,[25] commercially available 4-methoxy-3-pyrrolin-2-one (**21**) was subjected to a Vilsmeier−Haack reaction using $POBr_3$ and DMF, and the ensuing azafulvene **40** (49% yield) was engaged in a Suzuki−Miyaura cross-coupling reaction[34] with the readily available boronic acid **23** to give the required aldehyde **17** in 95% yield. The spectral data acquired on this aldehyde (**17**) were in complete accord with those reported by Lavallée and co-workers.[25]



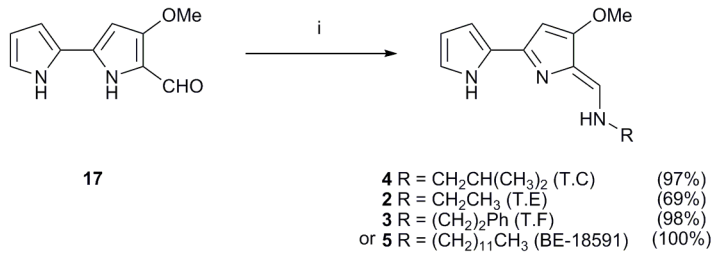
21 **40** **23** **17**

Scheme 1-6: *Reagents and conditions* (i) $POBr_3$, $Et_2NC(O)H$, $CHCl_3$, 0 °C, 0.5 h; 60 °C, 5 h; (ii) $Pd(PPh_3)_4$, Na_2CO_3, H_2O/1,4-dioxane, 100 °C, 2 h.

1.3.2 The synthesis of tambjamines C, E, F and BE-18591

With the aldehyde **17** in hand, its conversion into various target tambjamines could begin. Accordingly, reaction of aldehyde **17**, as a solution in 1,2-dichloroethane (DCE), with the relevant range of commercially available alkyl amines in the presence of acetic acid at 18−50 °C then afforded the acetate salts of tambjamines **2-5** in yields ranging from 69 to 100% (Scheme 1-7). For example, treatment of aldehyde **17** with 1-aminododecane under the specified conditions produced the acetate salt of BE-18591 (**5**) in 100% yield. The spectral data derived from this material were in complete accord with those reported for the natural product (Table

1-1).[1] Thus far, we have been unable to identify conditions under which aldehyde **17** reacts with ammonia or a surrogate thereof so as to provide useful quantities of the parent natural product, namely tambjamine A (**1**).



17

4 R = $CH_2CH(CH_3)_2$ (T.C) (97%)
2 R = CH_2CH_3 (T.E) (69%)
3 R = $(CH_2)_2Ph$ (T.F) (98%)
or **5** R = $(CH_2)_{11}CH_3$ (BE-18591) (100%)

Scheme 1-7: *Reagents and conditions* (i) RNH_2, AcOH, DCE, 50 °C, 2 h.

The NMR data recorded on synthetically- and naturally- derived tambjamines C, E, F and BE-18591 are tabulated in Appendix one. For the purposes of illustration, a comparison of the NMR data recorded for synthetically-derived tambjamine BE-18591 with those recorded on the naturally-derived material follows in Section 1.3.3.

1.3.3 Comparison of the NMR data recorded for the naturally- and synthetically-derived tambjamine BE-18591

Synthetically-derived BE-18591 (**5**), like other tambjamine samples prepared here, was isolated as its acetic acid salt. As expected, the acetate counter-anion gave rise to some extra signals in the associated ^{13}C and ^{1}H NMR spectra when compared to the spectra recorded on the naturally-derived free bases as published by Kojiri and co-workers (see Table 1-1).[1] The acetate counter-anion gave rise, as expected, to signals at δ 179.0 and 24.6 in the ^{13}C NMR spectrum, and to a singlet at δ 2.06 in the ^{1}H NMR spectrum. All sp^2-hybridized carbon nuclei appear in the range δ 163.2 and 91.0 and show a good correlation with those recorded on the naturally-derived material; the average deviation is 0.60 ppm and the greatest deviation is less than 1.0 ppm. The chemical shifts of the methoxy carbon and aminomethylenic carbon are also in close agreement. The chemical shift recorded for these moieties was δ 58.0 and 50.9, respectively, and these compare favourably with the values listed for the naturally-derived material; the deviation of these values from those listed in the literature are 0.4 and 0.1 ppm, respectively. The remaining carbon signals arise from the docecyl sidechain. In order to account for every carbon atom present in the framework of tambjamine **5**, it must be that two carbon signals overlap at around δ 29.5 in the spectrum recorded on the synthetic sample, though this is not surprising given the congestion of signals at this region. Where direct comparisons are possible, however, the chemical shifts recorded for the carbon nuclei constituting the dodecyl sidechain show a very close agreement with values obtained from the naturally-derived material; the average deviation is 0.10 ppm and the maximum deviation is 0.2 ppm.

As is typical for spectra recorded on tambjamines isolated as the acetic acid salts, the resonances due to the amino protons are coalesced in the ^{1}H NMR spectrum (at δ 11.4); this is likely due to an enhanced rate of exchange and equivalence effected by complexation of the molecule with acetic acid. Other characteristics of the ^{1}H NMR spectrum recorded on synthetically-derived BE-18591 (**5**) are in close accord with those reported by Kojiri *et. al*.[1] For instance, all signals expected to arise from protons of the dodecyl sidechain are present and integrate for the anticipated 25 protons. Where direct comparisons of chemical shifts are possible, the deviation between signals in the synthetically- and naturally-derived spectra does not exceed 0.1 ppm. Likewise pyrrole-bound protons bound to carbon show good correlations. The distribution of these signals is nearly identical in the two spectra, although those signals derived from the synthetic sample were consistently shifted slightly upfield, by 0.06 ppm on average.

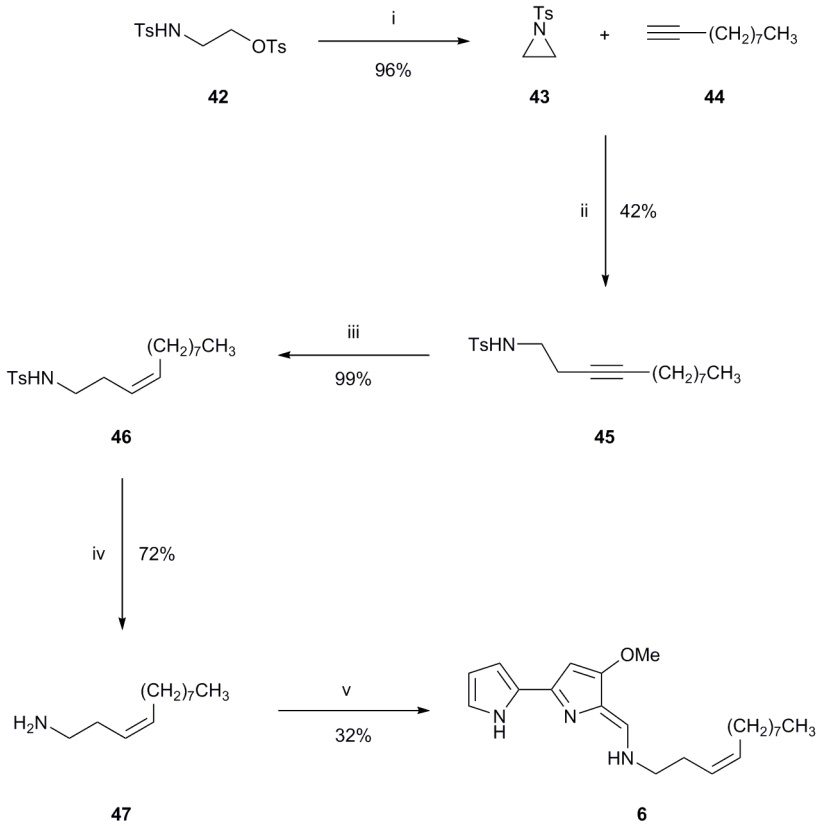
A strong absorption band, at 2923 cm^{-1}, was observed in the IR spectrum of the synthetic sample of tambjamine **5**. The same band was also observed in the naturally-derived material (at 2926 cm^{-1}) and it is likely arises from stretching of the imine C=N bond. The ESI mass spectrum of tambjamine **5** displays a $[M+H]^+$ ion at *m*/*z* 358, and accurate mass measurement on this species established the molecular formula of the compound is $C_{22}H_{35}N_3O$, as expected.

Table 1-1: *Comparison of the ^{13}C and ^{1}H NMR data recorded on naturally- and synthetically-derived BE-18591 (**5**), isolated as the free base and acetic acid salt, respectively.*

^{13}C NMR Data Natural **5** (δ_C) (75 MHz, $CDCl_3$)[35]	^{13}C NMR Data Synthetic **5** (δ_C) (75 MHz, $CDCl_3$)	^{1}H NMR Data Natural **5** (δ_H) (300 MHz, $CDCl_3$)[35]	^{1}H NMR Data Synthetic **5** (δ_H) (300 MHz, $CDCl_3$)
	179.0	10.8 (broad s, 1H)	11.4 (s, 2H)
163.6	163.2	9.50 (broad s, 1H)	
142.4	142.6	7.33 (broad d, *J* = 9.0 Hz, 1H)	7.26 (s, 1H)
140.0	140.5	7.06 (m, 1H)	7.00 (m, 1H)
124.1	123.4	6.74 (m, 1H)	6.64 (m, 1H)
122.3	123.1	6.28 (m, 1H)	6.21 (m, 1H)
113.2	112.3	5.96 (s, 1H)	5.89 (s, 1H)
110.6	110.9	3.93 (s, 3H)	3.83 (s, 3H)
110.6	109.9	3.47 (broad t, *J* = 7.0 Hz, 2H)	3.37 (t, *J* = 7.1 Hz, 2H)
91.9	91.0		2.06 (s, 3H, $CH_3CO_2^-$)
58.4	58.0	1.75 (m, 2H)	1.66 (m, 2H)
51.0	50.9	1.20-1.45 (m, 18H)	1.22 (m, 18H)
31.9	31.8	0.88 (t, *J* = 7.0 Hz, 3H)	0.85 (t, *J* = 6.8 Hz, 3H)
30.2	30.3		
29.6			
29.6	29.5		
29.6	29.4		
29.4	29.3		
29.3	29.2		
29.1	29.1		
26.5	26.4		
22.7	24.6		
	22.6		
14.1	14.0		

1.3.4 The synthesis of a tambjamine bearing an unsaturated side-chain

The unsaturated primary amine required for the preparation of tambjamine **6** has not been reported in the literature previously. Scheme 1-8 outlines the process by which *cis*-alkene **47** was obtained in four steps from *bis*-tosylethanolamine **42** and the subsequent condensation of the former compound with aldehyde **17** to afford tambjamine **6**.



Scheme 1-8: *Reagents and conditions* (i) KOH, H_2O; (ii) *n*-BuLi, THF; (iii) H_2, Lindlar's cat., quinoline, hexane, 18 °C, 2 h; (iv) $C_{10}H_8Na$, DME, 18 °C, 1 h; (v) AcOH, compound **17**, DCE, 18 °C, 2h.

Thus, following a protocol reported by Bulkowski,[36] the *bis*-tosyl derivative, **42**, of ethanolamine was treated with aqueous KOH to give aziridine **43**.[36] Using a procedure reported by Gronquist and Meinwald, this compound was then subjected to nucleophilic ring-opening with the anion derived from 1-decyne (**44**).[37] Each of the seventeen chemically unique carbons present in alkyne **45** was observed in the ^{13}C NMR spectrum recorded on this compound. Resonances arising from the two alkynyl carbons appeared at δ 83.2 and 75.6.

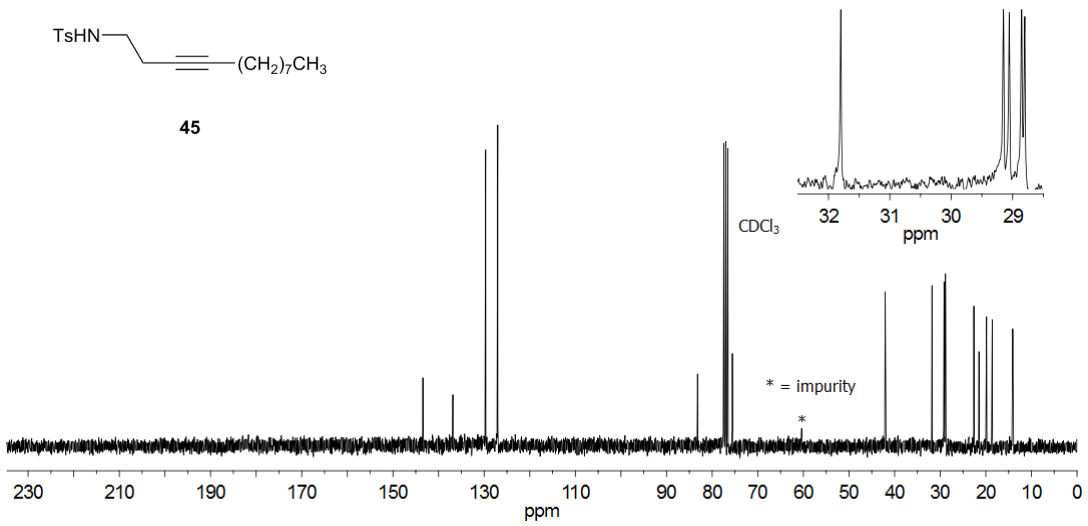


Figure 1-4: *75 MHz ^{13}C NMR spectrum of alkyne **45** (recorded in $CDCl_3$).*
Inset: An expansion of a region from the spectrum.

Internal alkyne **45** was subjected to hydrogenation using Lindlar's catalyst in the presence of quinoline[38] so as to afford the *Z*-alkene **46** in 99% yield. The ^{1}H NMR spectrum recorded on *cis*-alkene **46** exhibited multiplets at δ 5.49 and 5.15 that were assigned to the pair of alkenic protons introduced in the catalytic hydrogenation reaction. In most other respects, this spectrum bore a close resemblance to that of the precursor alkyne. The ESI mass spectrum of alkene **46** exhibited an $[M+H]^+$ ion at *m/z* 338 and an accurate mass measurement of this species established that it possessed the expected molecular formula, *viz.* $C_{19}H_{31}NO_2S$. The derived ^{13}C NMR spectrum exhibited fifteen signals, rather than the expected seventeen, and this implied that some of the signals overlap.

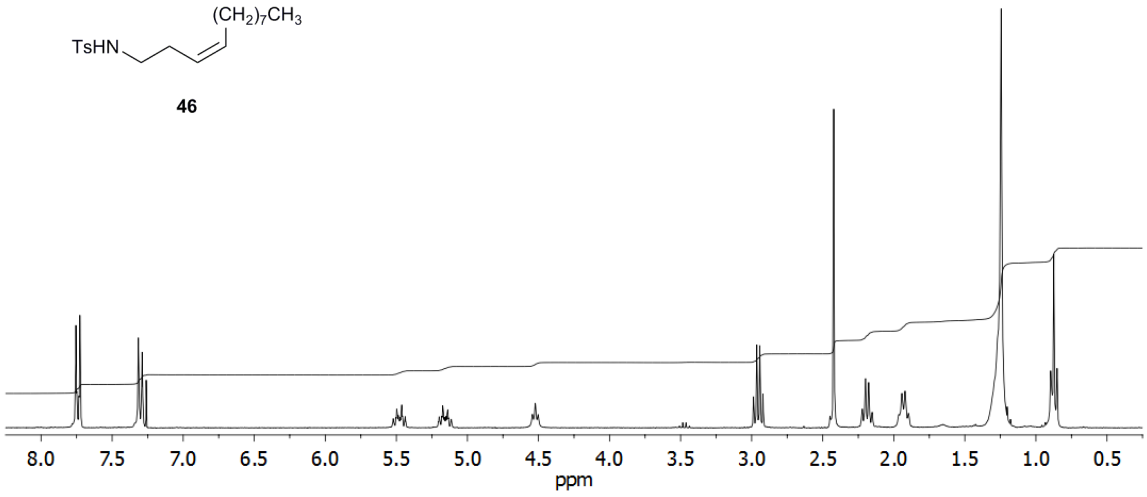


Figure 1-5: *300 MHz ^{1}H NMR spectrum of* cis*-alkene **46** (recorded in $CDCl_3$).*

Reductive cleavage of the sulfonamide linkage within *cis*-alkene **46** using sodium naphthalenide in 1,2-dimethoxyethane (DME)[39] then afforded the required amine **47**. Consistent with this

transformation, proton resonances in the aromatic region of the ^{1}H NMR spectrum of compound **47** were no longer present indicating that the primary amine had been successfully obtained from its *N*-tosyl precursor. The infrared spectrum of compound **47** showed a broad absorption band at 3431 cm^{-1} that is attributed to N−H bond stretching. Associated N−H bending resulted in the appearance of an absorption band at 1577 cm^{-1}.

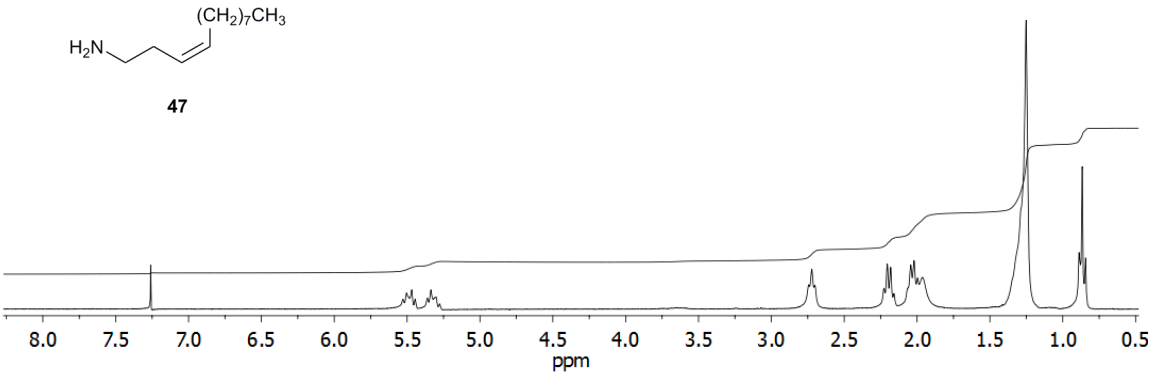


Figure 1-6: *300 MHz ^{1}H NMR spectrum of primary amine **47** (recorded in $CDCl_3$).*

Amine **47** was then engaged in the, by now, standard condensation reaction with aldehyde **17** to give tambjamine **6**. The ^{1}H and ^{13}C NMR spectra derived from compound **6** match those recorded on the natural product.[2] Furthermore, the infrared and mass spectral data obtained on the synthetic material were in full accord with the assigned structure.

Tambjamine **6**, isolated as its acetic acid salt from the condensation of amine **47** with aldehyde **17**, exhibited spectral properties that were in close agreement with those reported for the natural product isolated by Kumar and co-workers.[2] The ^{13}C NMR spectrum is shown in Figure 1-7.

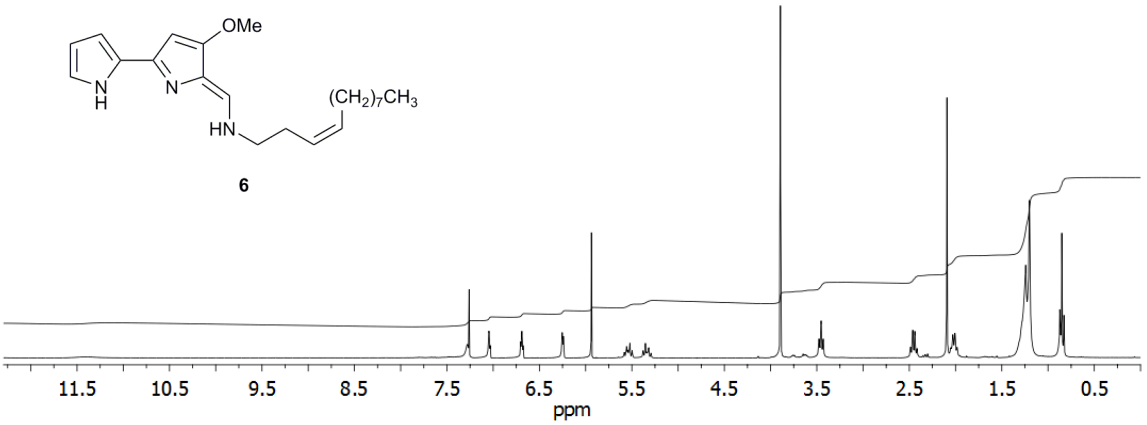


Figure 1-7: *300 MHz ^{1}H NMR spectrum of tambjamine **6** (recorded in $CDCl_3$).*

For instance, the pair of multiplets arising from the *Z*-alkene embedded within the twelve-carbon side-chain was observed at δ 5.54 and 5.34. In the publication that describes the isolation of this compound and the assignment of NMR resonances, these same signals appear (though

each as discernable doublets of triplets) at δ 5.55 and 5.35, respectively. (See Table 1-2). The ESI mass spectrum of tambjamine **6** exhibited a molecular ion at *m/z* 356 and an accurate mass measurement carried out on this species established that it possessed the molecular formula ($C_{22}H_{32}N_3O$) expected for tambjamine **6**.

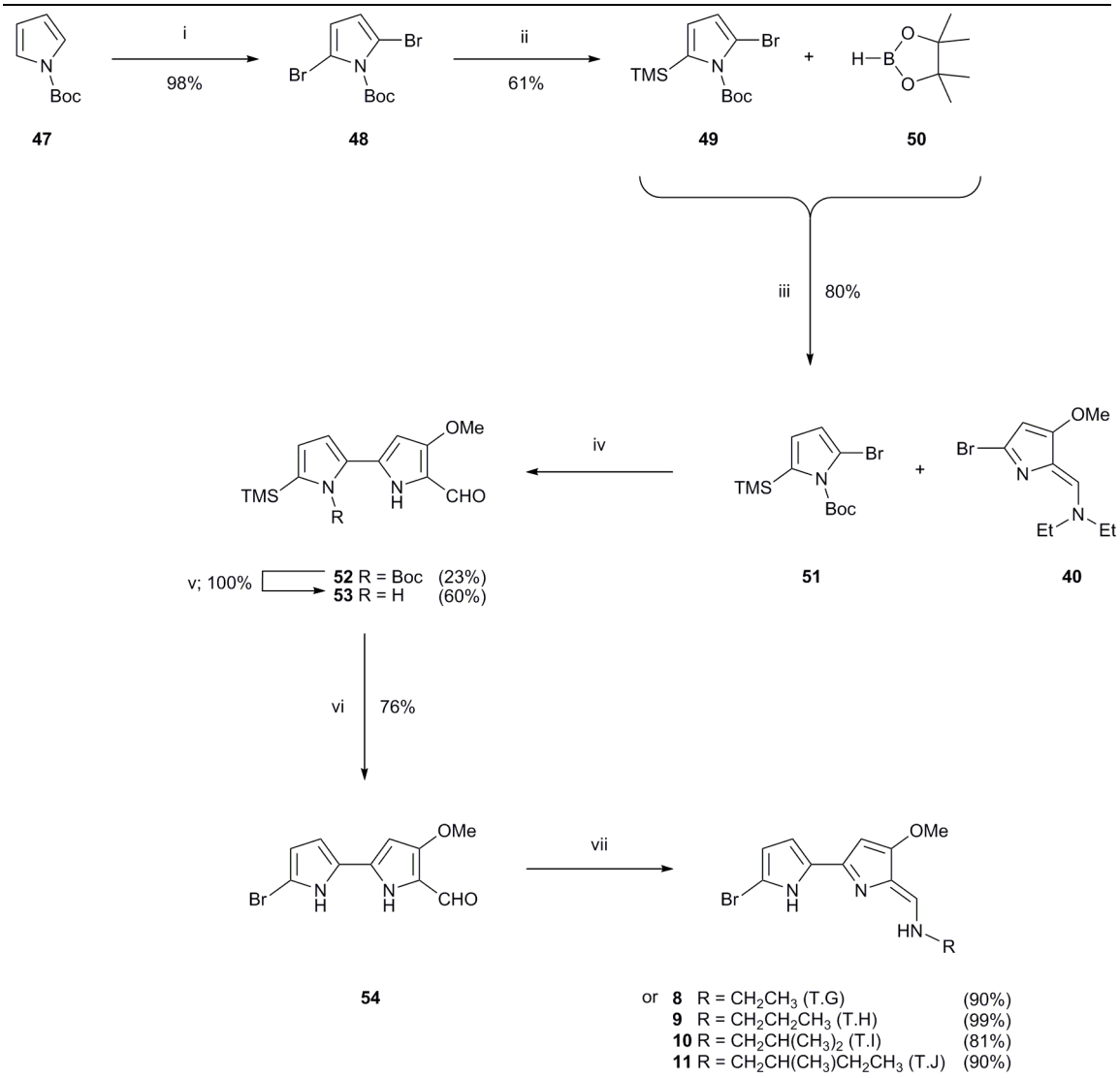
Table 1-2: *Comparison of the ^{13}C and ^{1}H NMR data recorded on naturally- and synthetically-derived tambjamine **6**, isolated at the free base and acetic acid salt respectively.*

^{13}C NMR Data Natural **6** (δ_C) (75 MHz, $CDCl_3$)[2]	^{13}C NMR Data Synthetic **6** (δ_C) (75 MHz, $CDCl_3$)	^{1}H NMR Data Natural **6** (δ_H) (300 MHz, $CDCl_3$)[2]	^{1}H NMR Data Synthetic **6** (δ_H) (300 MHz, $CDCl_3$)
	179.0		11.4 (s, 2H)
163.9	163.5	7.29 (d, *J* = 14 Hz, 1H)	7.28 (s, 1H)
143.4	142.9	7.06 (m, 1H)	7.04 (m, 1H)
141.1	140.6	6.70 (m, 1H)	6.69 (m, 1H)
134.5	134.1	6.25 (m, 1H)	6.25 (m, 1H)
124.1	123.9	5.95 (s, 1H)	5.94 (s, 1H)
124.0	123.7	5.55 (dt, *J* = 10.8 Hz, 1H)	5.54 (m, 1H)
123.5	123.1	5.35 (dt, *J* = 10.8 Hz, 1H)	5.34 (m, 1H)
113.0	112.5	3.90 (s, 3H)	3.89 (s, 3H)
111.3	110.9	3.46 (m, 2H)	3.45 (t, *J* = 6.9 Hz, 2H)
110.5	110.1	2.46 (m, 2H)	2.45 (m, 2H)
91.6	91.2		2.09 (s, 3H, $CH_3CO_2^-$)
58.8	58.2	2.01 (m, 2H)	2.02 (m, 2H)
51.2	50.8	1.70-1.21 (complex m, 12H)	1.22 (m, 12H)
28.8	31.8	0.86 (t, 3H)	0.85 (t, *J* = 6.8 Hz, 3H)
27.8	29.7		
27.7-26.5 (6C)	29.5		
	29.3		
	29.3		
	28.4		
	27.4		
	24.5		
	22.6		
14.5	14.1		

1.3.5 The synthesis of 4-methoxy-5'-bromo-2,2'-bipyrrole-5-carboxaldehyde

Our initial attempts to generate the requisite bromo-analogues of compound **17**, for use in the preparation tambjamines **7**−**11**, involved treating the aldehyde **17** with various electrophilic brominating agents. Unfortunately, under all the conditions investigated thus far only complex mixtures of products were obtained. Accordingly, directed syntheses of such compounds were

pursued. Eventually, the route shown in Scheme 1-9 was established as a method for obtaining the 5′-bromo-analogue, **54**, of compound **17**.



Scheme 1-9: *Reagents and conditions* (i) NBS, THF, −78 °C, 2 h; (ii) *n*-BuLi, THF, −78 °C → 0°C, 0.5 h, TMS−Cl, −78 °C → 0 °C, 0.5 h; (iii) $PdCl_2$·dppf·CH_2Cl_2, Et_3N, 1,4-dioxane, 80 °C, 18 h; (iv) $Pd(PPh_3)_4$, Na_2CO_3, toluene, water, 1,4-dioxane, 100 °C, 15 h; (v) *m*-xylene, 139 °C, 1 h; (vi) Pyr·HBr_3, THF, −78 °C → 18 °C, 2 h; (vii) RNH_2, AcOH, DCE, 18 °C, 4 h.

Thus, following protocols established by Weinreb *et al.*,[40] the readily available *N*-Boc protected pyrrole **47** was subjected to two-fold bromination with *N*-bromosuccinimide (NBS) and the ensuing 2,5-dibrominated derivative **48** was treated with *n*-BuLi and then trimethylsilyl chloride (TMS-Cl). In this manner the *C*-silylated compound **49**[40] was obtained in 61% yield. Miyaura borylation of this material,[34] using borane **50**, then afforded the pyrrole **51** that could be engaged in a Suzuki-Miyaura cross-coupling reaction with azafulvene **40** so as to yield a chromatographically separable mixture of compounds **52** (23%) and **53** (60%). Heating the former product in refluxing xylene effected thermolytic cleavage of the Boc-group and delivered further quantities of the TMS-derivative **53** (100%) of the aldehyde **17**. The spectral

data derived from compound **53** were in full accord with the assigned structure but final confirmation of this followed from a single-crystal X-ray analysis. The derived ORTEP diagram is shown in Figure 1-8. Surprisingly, the silylated pyrrole **53** failed to engage in a clean *ipso*-substitution reaction upon exposure to NBS. In contrast, reaction of substrate **53** with freshly prepared pyridinium hydrobromide perbromide[41] led to the desired compound, **54**, in 76% yield.

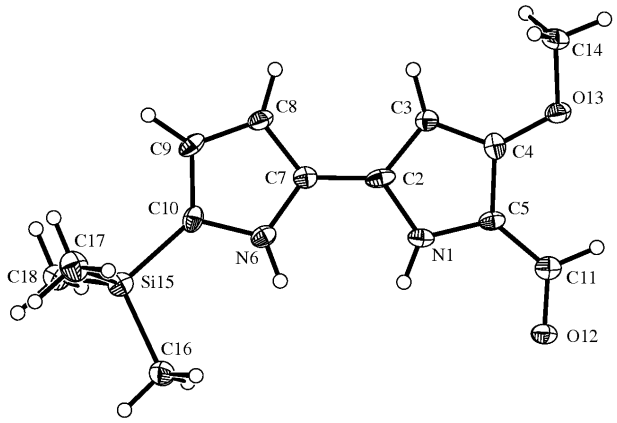


Figure 1-8: *ORTEP derived from the single-crystal X-ray analysis of aldehyde **53**.*

1.3.6 The synthesis of tambjamines G, H, I and J

Following the protocols described above, compound **54** was condensed with the relevant range of primary alkyl amines, in the presence of acetic acid. These conditions then afforded, after conventional flash chromatographic purification on silica gel, the acetate salts of tambjamines **8**–**11** in yields ranging from 81 to 99%. (See Scheme 1-9). The spectral data derived from each of these materials matched those reported for the corresponding natural products, some of which have also been isolated as their acetate salts.[42] Tabulation of the NMR data recorded on naturally- and synthetically-derived tambjamines G, H, I and J is included in Appendix One (page 175). A brief description of the spectral data derived from tambjamine J (**11**), which is typical of these four brominated 5′-brominated tambjamines, follows.

The ^{1}H NMR spectrum recorded on tambjamine J (**11**) is shown in Figure 2-6. The AA′XY system arising from the diasteriotopic methylene protons appears at 3.28 ppm. The chemical shifts of the signals observed in the ^{1}H NMR spectrum of synthetically-derived tambjamine J (**11**) are in close accord with those recorded on the naturally-derived material (See Table 1-3). For instance, all signals expected to arise from protons on the 2-methylbutyl sidechain are present and integrate for the anticipated 11 protons. The chemical shifts of pyrrole-bound C–H protons show good correlations between the two spectra. The distribution of these signals is nearly identical for the two spectra, though those signals derived from the synthetic sample are consistently shifted slightly upfield, by 0.04 ppm on average.

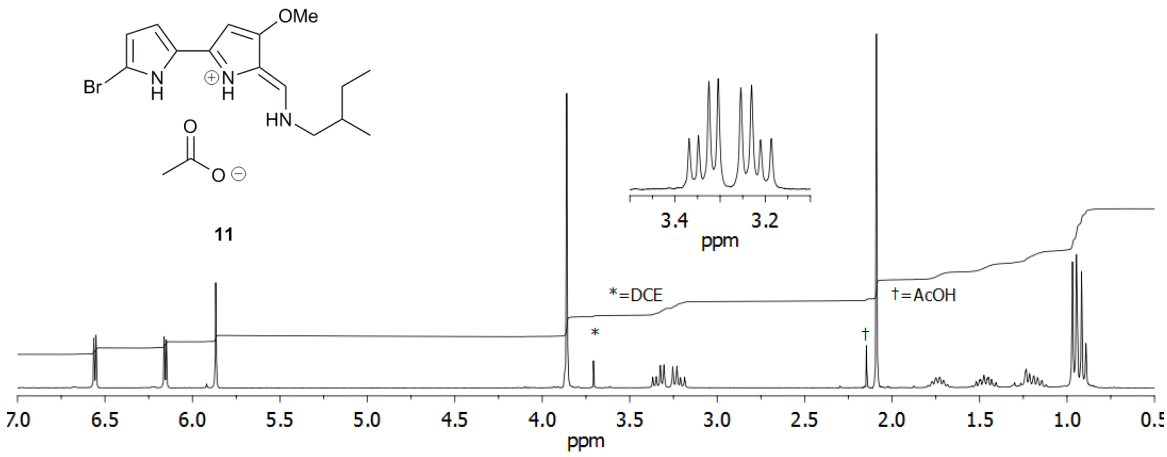


Figure 1-9: *300 MHz ^{1}H NMR spectrum of tambjamine **11** (recorded in $CDCl_3$).*
Inset: An expansion of a region from the spectrum.

The ^{13}C spectra recorded for synthetically-derived tambjamine J (**11**), which displays the expected seventeen signals, agrees closely with that reported by Blackman *et al.*[42] for a naturally-derived sample of the same material (Table 1-3). The average deviation between the signals recorded in each spectrum was 0.72 ppm with the largest difference being 0.90 ppm. (See Table 1-3).

The EI mass spectrum displays a pair of molecular ion at *m*/*z* 339 and 337. the 1:1 ratio of these species is as would be expected for a monobrominated compound. An accurate mass measurement on the lighter species established that it was of the expected molecular composition, *viz*. $C_{15}H_{20}{}^{79}BrN_3O$.

Table 1-3: *Comparison of the ^{13}C and ^{1}H NMR data recorded on naturally- and synthetically-derived tambjamine J (**11**), both isolated as the corresponding acetic acid salt.*

^{13}C NMR Data Natural **11** (δ_C) (75 MHz, $CDCl_3$)[42]	^{13}C NMR Data Synthetic **11** (δ_C) (75 MHz, $CDCl_3$)	^{1}H NMR Data Natural **11** (δ_H) (300 MHz, $CDCl_3$)[42]	^{1}H NMR Data Synthetic **11** (δ_H) (300 MHz, $CDCl_3$)
179.0	179.3	13.20 (broad s, 1H)	11.10 (broad s, 2H)
164.3	163.4	11.10 (broad s, 1H)	
142.3	141.5	7.30 (broad s, 1H)	7.26 (s, 1H)
142.1	141.3	6.63 (d, *J* = 4.2 Hz, 1H)	6.56 (d, *J* = 3.9 Hz, 1H)
125.1	124.4	6.23 (d, *J* = 4.2 Hz, 1H)	6.15 (d, *J* = 3.9 Hz, 1H)
114.3	113.5	5.90 (s, 1H)	5.87 (s, 1H)
113.0	112.1	3.90 (s, 3H)	3.86 (m, 3H)
111.7	110.9	3.33 (m, 2H)	3.28 (m, 2H)
106.0	105.3	2.14 (m, 1H)	
91.5	90.8	2.10 (s, 3H, $CH_3CO_2^-$)	2.09 (s, 3H, $CH_3CO_2^-$)
58.9	58.2		1.73 (m, 1H)
57.8	57.0	1.56 (m, 2H)	1.46 (m, 1H)
36.6	35.8		1.19 (m, 1H)
27.2	26.5	1.30 (m, 3H)	0.93 (m, 6H)
24.1	24.6	0.98 (t, *J* = 8.0 Hz, 3H)	
17.4	16.7		
11.8	11.1		

1.4 BIOLOGICAL EVALUATION OF SYNTHETICALLY-DERIVED TAMBJAMINES C, E, F, G, H, I AND J, BE-18591 AND A RELATED ALKALOID FROM THE MARINE BACTERIUM *PSEUDOALTEROMONAS TUNICATA*

1.4.1 Introduction

Natural products continue to provide a rich source of new drugs[43-48] with those being isolated from organisms found in marine and freshwater environments becoming increasingly prominent in this regard.[49-55] For example, micro-organisms, including those found in marine ecosystems, provide a remarkable array of biologically active metabolites.[56] In those instances where limited quantities of metabolites have been available from the natural source, synthesis techniques have often been deployed to address the problem of supply and thus provide sufficient material for extended biological evaluations.[57-60] It is rare, however, that such techniques have been used to deliver samples of almost every member of an entire class of natural product for the purpose of carrying out their comprehensive biological evaluation. This section details such a case. Specifically, this evaluation of synthetically-derived samples of tambjamines **2**–**4**, **8**–**11**, BE-

18591 (**5**) and pyrrole **6** as antimicrobial and cytotoxic agents was conducted by colleagues at the Federal University of Ceará in Brazil and at the University of New South Wales, Australia. The outcomes of these studies are described in below.

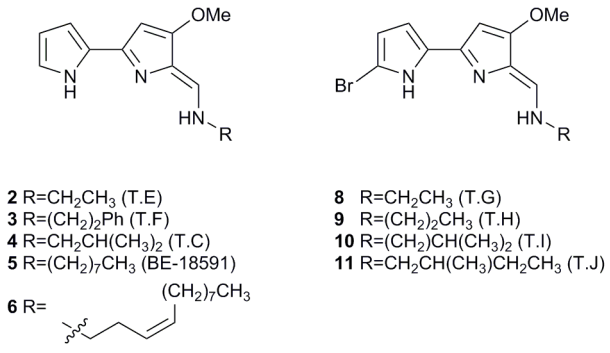


Figure 1-10: *Synthetically-derived tambjamines that were evaluated for biological activity.*

The first total syntheses of seven of the ten members of the tambjamine class of natural product and analogous preparation of BE-18591 (**5**) and congener **6** by the methods described earlier meant that a significant proportion of the members of the entire tambjamine class became available for biological screening and thus offered the prospect of developing a structure-activity relationship (SAR) profile for this family of marine natural product.[28] Details of the outcomes of some such screening regimes are presented below.

1.4.2 Antimicrobial screening

Each of the tambjamines **2**–**6** and **8**–**11** was tested at 0.1 and 1.0 mg/ml concentrations against *Candida albicans*, *Malassezia furfur*, *Escherichia coli* and *Staphylococcus aureus* using a standardized paper disc-agar diffusion method (Bauer and Kirby method).[61] The potent and clinically-employed antifungal agent amphotericin B was used as a positive control and the observed diameters of the zones of inhibition are presented in Table 1-4.

Table 1-4: *Zones of Inhibition Observed During Testing of Tambjamines **1-9** Against* C. albicans, M. furfur, E. coli *and* S. aureus *Using the Kirby–Bauer or Disc Diffusion Method* [a]

	C. albicans		*M. furfur*		*E. coli*		*S. aureus*	
Compound [mg/ml]	0.1	1.0	0.1	1.0	0.1	1.0	0.1	1.0
2	– [b]	7	+ [c]	20	–	18	–	19
3	–	+	–	+	–	9	–	9
4	–	+	12	15	–	13	–	20
8	–	+	16	18	–	13	–	20
9	–	8	15	17	–	14	–	19
10	–	–	17	>17	–	14	8	20
11	–	–	17	>17	–	11	9	20
5	–	11	+	>16	–	9	8	10
6	–	10	8	>16	–	8	+	8
Amphotericin B	13	NT [d]	+	NT	–	NT	–	NT
Methanol [e]	–	–	–	–	–	–	–	–

[a] Measured in mm. [b] – = No detectable inhibition. [c] + = Detectable but modest inhibition. [d] NT = Not tested. [e] Methanol was used as negative control.

The results of this antimicrobial screening regime reveal that all of compounds **2**–**6** and **8**−**11** display antibacterial and antifungal effects although at lower concentrations they showed poor activity against *E. coli* and *S. aureus* and were selectively active against *M. furfur* over *C. albicans*. Interestingly, this activity against *M. furfur* (a dandruff-causing organism) was higher than that exhibited by amphotericin B. As such the tambjamines could have promising applications in this area provided, *inter alia*, issues surrounding the cost of production can be overcome.

1.4.3 Biological screening against certain human cancer cell lines

Our objectives in this area were two-fold: (i) to evaluate the cytotoxic potential of compounds **2**–**6** and **8**−**11** against four human cancer cell lines as well as against (normal) human peripheral blood mononuclear (PBM) cells and, (ii), to study the underlying molecular mode of cytotoxic action of the tambjamines using human promyelocytic leukemia cells (HL-60), a cell line particularly suited to examining cell proliferation, cell-cycle and apoptotic events.[62-64]

The outcomes of the testing of compounds **2**–**6** and **8**−**11** against the human cancer cell lines HL-60 (promyelocytic leukemia cells), MDA-MB-435 (derived from the M14 melanoma cell line), HCT-8 (ileocecal colorectal adenocarcinoma cell line) and SF-295 (glioblastoma cell line) as well as PBM cells are presented in Table 1-5. The potent anti-cancer agent doxorubicin (Adriamycin™) was used as a positive control. Given the nature of the test protocols used (see Experimental), only compounds showing IC_{50} values of less than 1 μg/ml in at least one cell line were considered active. On this basis, then, and with the exception of compound **3**, the tambjamines and their congeners described herein can be regarded as rather potent cytotoxic

agents although most were less active than doxorubicin. Furthermore, none of them was especially selective for the cancer cell lines when compared to their effects on PBM cells.

Table 1-5: *Cytotoxic Effects of Compounds **2–6** and **8−11** on Cancer and PBM Cell Lines* [a]

	Cancer cell lines				
	HL-60	MDA-MB435	HCT-8	SF-295	PBM
Compound			IC_{50} (CI 95%)		
2	0.70 (0.59–0.82)	3.42 (2.59–4.52)	1.41 (1.25–1.59)	1.64 (1.45–1.86)	2.53 (1.74–3.68)
3	>25 (ND [b])	>25 (ND)	>25 (ND)	>25 (ND)	>25 (ND)
4	0.80 (0.67–0.95)	2.27 (1.78–2.91)	1.04 (0.83–1.30)	1.08 (0.87–1.35)	3.25 (2.38–4.37)
8	0.82 (0.75–0.88)	1.19 (0.98–1.44)	0.65 (0.57–0.73)	0.59 (0.50–0.69)	2.10 (1.45–3.04)
9	2.12 (1.87–2.41)	2.57 (2.26–2.93)	2.46 (2.07–2.92)	1.31 (1.15–1.48)	4.16 (3.52–4.93)
10	0.61 (0.49–0.77)	0.83 (0.71–0.96)	0.52 (0.46–0.59)	0.41 (0.35–0.48)	1.11 (0.83–1.50)
11	0.49 (0.41–0.60)	0.61 (0.52–0.72)	0.35 (0.32–0.38)	0.35 (0.28–0.43)	0.77 (0.55–1.08)
5	0.23 (0.18–0.31)	0.46 (0.34–0.63)	0.54 (0.43–0.68)	0.39 (0.33–0.47)	0.46 (0.27–0.80)
6	0.69 (0.60–0.80)	1.11 (0.95–1.30)	0.98 (0.85–1.12)	0.83 (0.73–0.94)	0.64 (0.31–1.30)
Dox [c]	0.02 (0.01–0.02)	0.48 (0.34–0.66)	0.23 (0.19–0.25)	0.04 (0.03–0.05)	0.23 (0.02–0.56)

[a] Data are presented as IC_{50} [μg/ml]. The 95% confidence intervals (CI 95%) shown were obtained by a non-linear regression analysis for all cell lines from three independent experiments. [b] ND = Not determined. [c] Doxorubicin (Dox) was used as positive control.

When compounds **2**–**6** and **8**−**11** were tested on mouse erythrocytes (red blood cells), which are non-nucleated cells in their mature form, they displayed little or no activity (i.e. no hemolytic properties) except those (*viz*. **5** and **6**) bearing the longer lipophilic attached to the (1*Z*)-1-[3-methoxy-5-(1*H*-pyrrol-2'-yl)-2*H*-pyrrolylidene]-methanamine core (Table 1-6). Given that these activities of compounds **5** and **6** can probably be attributed to their detergent effects, the cytotoxic profiles of the tambjamines as revealed in Table 1-5 could well arise through their interactions with nuclear DNA.

Table 1-6: *Cytotoxic Effects of Compounds **2–6** and **8−11** on Mouse Erythrocytes After 1 and 2 h Periods of Incubation* [a]

Compound	EC_{50} (CI 95%)	
	1 h	2 h
2	>200 (ND [b])	>200 (ND)
3	>200 (ND)	>200 (ND)
4	>200 (ND)	>200 (ND)
8	>200 (ND)	>200 (ND)
9	>200 (ND)	>200 (ND)
10	>200 (ND)	>200 (ND)
11	>200 (ND)	>200 (ND)
5	>200 (ND)	150.8 (127.6–178.2)
6	40.3 (29.7–54.6)	36.9 (31.3–43.5)

[a] Data are presented as EC_{50} [μg/ml]. The 95% confidence intervals (CI 95%) shown were obtained by a non-linear regression analysis for all cell lines from three independent experiments. [b] ND = Not determined.

As part of initial efforts to understand the mechanism of cytotoxicity of the tambjamines, compounds **10** and **11** were chosen for further biological evaluation. In particular, a study was conducted so as to examine whether the cytotoxic activity of such compounds involves the inhibition of DNA synthesis and the induction of apoptosis. Since HL-60 cells are considered particularly good models for such studies,[62-64] cell viability was determined by a trypan blue dye exclusion test[65] after incubation of the HL-60 cells (3×10^5 cells/ml) with compounds **10** and **11**. Flow cytometric techniques were used to analyze the outcomes of such experiments. As shown in Figure 1-11, both compounds reduced cell viability significantly (and, in the case of **11**, rapidly) at 0.3 and 0.6 μg/ml concentrations, although not quite to the same extent as the positive control doxorubicin.

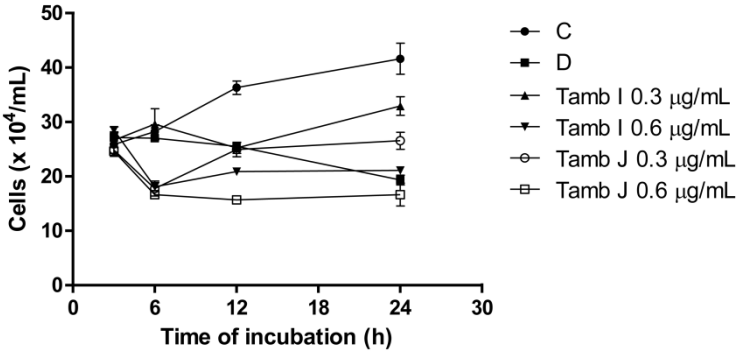


Figure 1-11: *Effect of tambjamines I (**10**) and J (**11**) on HL-60 cell viability as determined by trypan blue staining after 3, 6, 12 and 24 h of incubation.* Negative control (C) was treated with the vehicle used for diluting the tested substance. Doxorubicin (D, 0.3 μg/ml) was used as positive control. Results are expressed as mean ± standard error of measurement (SEM) from three independent experiments. *P <0.05 compared to control.

An analysis of the cell-death pattern in HL-60 cells caused by contact with compounds I (**10**) and J (**11**) was carried out using acridine orange (AO) and ethidium bromide (EB) staining techniques and then examining the resulting cell populations with a fluorescence microscope.[66]

The various colorations observed allow the cells within the population to be classified as viable (live), apoptotic or necrotic (dead).[67,68] The percentages of the latter two types of cell were then calculated after 3, 6, 12 and 24 h and the results of such studies (Figure 1-12) reveal that tambjamines **10** and **11** lead to cell death largely through an apoptotic pathway (at least under the conditions used here). Once again, however, these compounds were not quite as effective as the positive control doxorubicin in triggering this process.

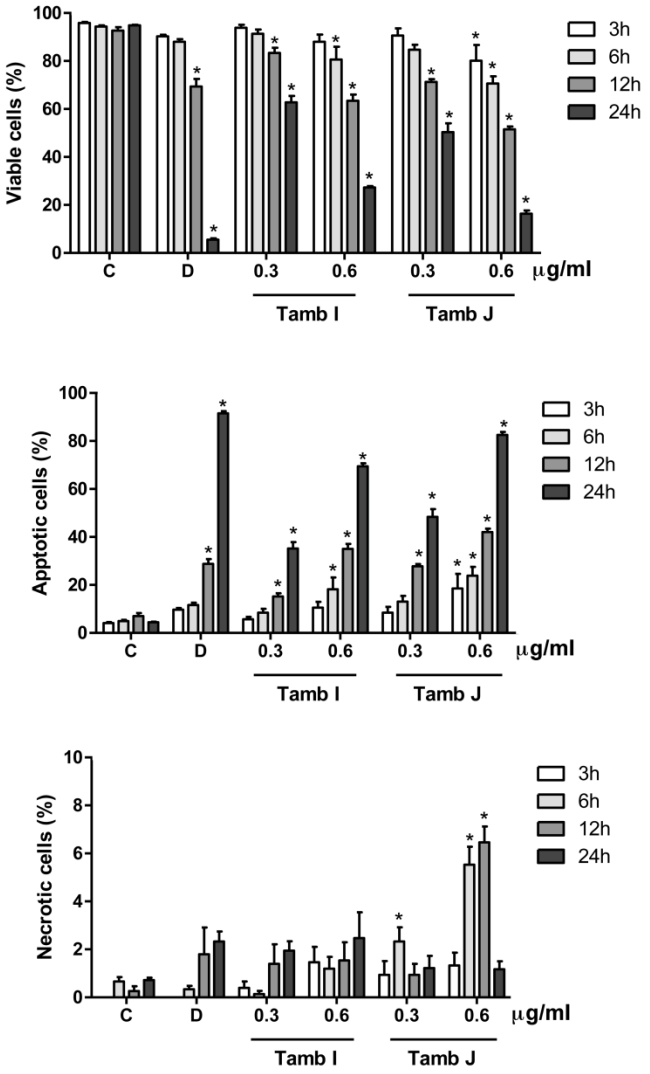


Figure 1-12: *Effect of tambjamine I (**10**) and J (**11**) on the HL-60 cell death pattern as determined by AO/EB-staining after 3, 6, 12 and 24 h of incubation*. Negative control (C) was treated with the vehicle used for diluting the tested substance. Doxorubicin (D, 0.3 μg/ml) was used as positive control. Results are expressed as mean ± standard error of measurement (SEM) from three independent experiments. *P <0.05 compared to control.

In a further effort to unravel the mode of cytotoxic action of the tambjamines, the impact of compounds **10** and **11** on the integrity of the plasma membrane of HL-60 cells was evaluated. Since the integrity of this membrane is preserved in cells undergoing apoptosis most of its functions remain unchanged, including the ability of the membrane to exclude dyes such as propidium iodide (PI). In contrast, those cells undergoing necrosis lose membrane integrity and

function very early and cannot, therefore, prevent the inclusion of PI. The results of carrying out the relevant test[69,70] are shown in Figure 1-13A and these establish that cell membrane integrity is preserved to a high degree (>80%) even in cases where the HL-60 cells have been exposed to 0.6 μg/ml concentrations of tambjamine **10** or **11**.

Since there are no marked changes in energy metabolism associated with apoptotic cells, the morphology of mitochondria in these systems remains unchanged. This is in contrast to necrosis, where mitochondrial swelling is one of the earliest changes. Accordingly, the mitochondrial transmembrane potential is preserved in apoptotic but not necrotic cells. This potential can be assayed by the capacity of the cell to retain the cationic probe rhodamine 123.[67,68] The outcomes of the relevant tests are shown in Figure 1-13B and reveal that less than 10% of the tambjamine-treated HL-60 cells have a low membrane potential ($\Delta\Psi m$) while more than 30% of the doxorubicin-treated cells do.

The death of cells undergoing apoptosis is preceded by chromatin cleavage at the linker regions between nucleosomes by specific endonucleases which results in extensive fragmentation of the DNA into oligonucleosomal subunits.[71] Accordingly, the percentage of DNA fragmentation observed in tambjamine-treated cells provides yet another measure of the extent of these compounds' apoptotic effects. Using nuclear fluorescence/flow cytometry methods as described by Nicoletti *et al.*,[71] the extent of DNA fragmentation of HL-60 cells was determined under the relevant set of conditions and the outcomes of such studies are shown in Figure 1-13C. Thus, >60% of DNA fragmentation was observed for both doxorubicin- and tambjamine-J-treated cells although in the latter case such levels were only observed after 24 h of incubation and when 0.6 μg/ml concentrations of the test compound, **11**, were used. DNA fragmentation levels of >40% were observed when compound **10** was used at the same concentration. Furthermore, tambjamines I and J did not promote changes in the cell cycle under the conditions used.

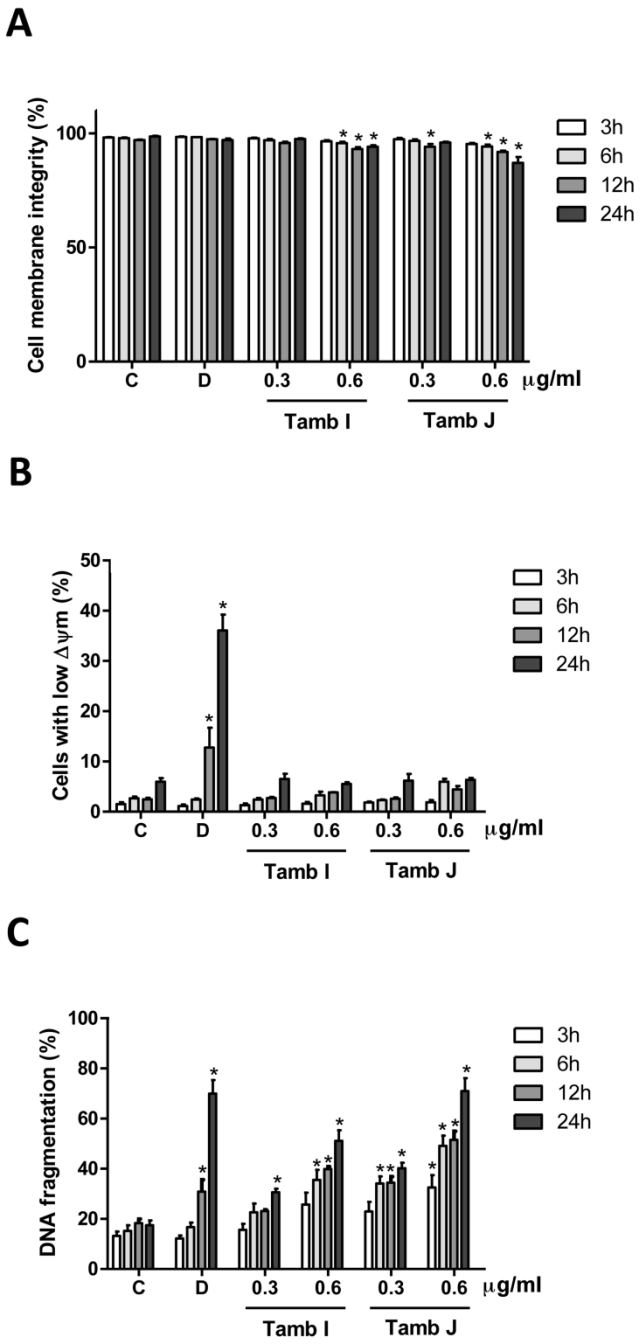


Figure 1-13: *Effect of tambjamines I (**10**) and J (**11**) on HL-60 cells. A = Effect on membrane integrity (as determined by a PI exclusion test). B = Effect on mitochondrial transmembrane potential (ΔΨm) (as determined by incorporation of rhodamine 123). C = Effect on DNA fragmentation (as determined by PI-induced nuclear fluorescence).* Negative control (C) was treated with the vehicle used for diluting the tested substance. Doxorubicin (D, 0.3 µg/ml) was used as positive control. Results are expressed as mean ± standard error of measurement (SEM) from three independent experiments. *P <0.05 compared to control.

Given the pivotal role that caspases play in apoptosis,[72-74] the effect of tambjamines I (**10**) and J (**11**) on the activation of such enzymes in HL-60 cells was measured after 24 h of incubation (Figure 1-14). This revealed that both compounds activate caspases 3/7 and 8 although these were not quite as those strong as those observed with doxorubicin. Given their effect on the last

enzyme, compounds **10** and **11** most likely trigger apoptosis *via* the so-called death receptor or extrinsic pathway.

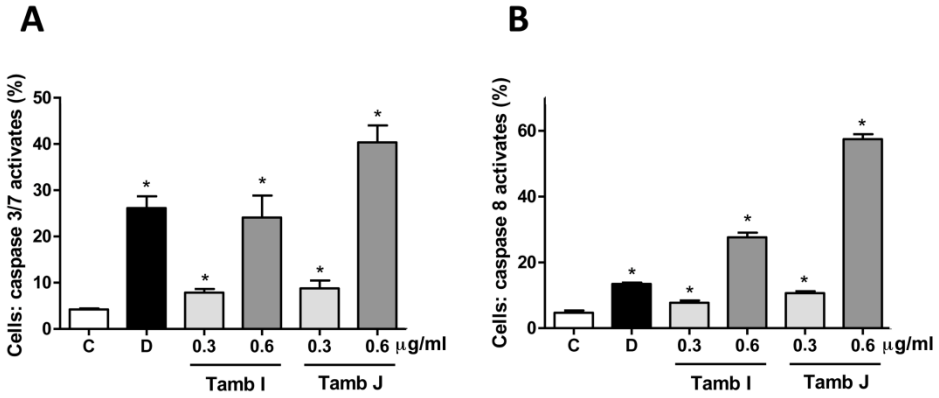


Figure 1-14: *Effect of tambjamines I (**10**) and J (**11**) on caspase 3/7 and 8 activation after 24 h of incubation. A = Caspase 3/7 activation. B = Caspase 8 activation.* Negative control (C) was treated with vehicle used for diluting the tested substances. Doxorubicin (D, 0.3 µg/ml) was used as positive control. Results are expressed as mean ± standard error of measurement (SEM) from three independent experiments. *P <0.05 compared to control.

In a further probing of the origins of the cytotoxic effects of compounds **10** and **11**, a comet assay[75] was used to detect DNA strand breaks and alkali-labile lesions within the HL-60 cells exposed to the drug for 24 h. The outcomes of this assay (Figure 1-15) reveal that, while the DNA damage index (DI – arbitrary units used) associated with the tambjamine-treated cells is not as high as that associated with those that had been exposed to doxorubicin, the natural products are clearly effecting DNA damage.

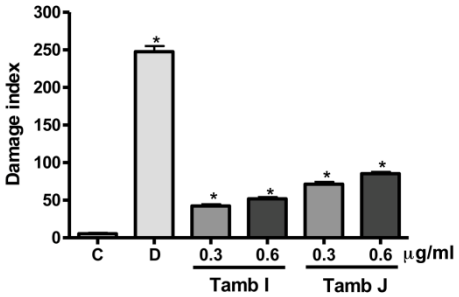


Figure 1-15: *Effect of tambjamine I (**10**) and J (**11**) on HL-60 cells DNA damage index after 24 h treatment.* Negative control (C) was treated with the vehicle used for diluting the tested substance. Doxorubicin (D, 0.3 µg/ml) was used as positive control. Results are expressed as mean ± standard error of measurement (SEM) from two independent experiments. *P <0.05 compared to control.

1.5 CONCLUSIONS AND FUTURE WORK

The tambjamines **2**–**6** and **8**–**11** clearly display useful antimicrobial and cytotoxic effects with the more lipophilic members of the series seeming to be the more potent in both settings. While none of the more cytotoxic compounds was especially selective for the cancer cell lines, the use of targeted drug delivery regimes[76] could be used to make them so. The prospects of achieving

this through appropriate functionalization of the alkyl chain attached to the (1*Z*)-1-[3-methoxy-5-(1*H*-pyrrol2'-yl)-2*H*-pyrrolylidene]methanamine core would seem rather good.

An investigation of the origins of the cytotoxic properties of the tambjamines indicates that they are genotoxic, causing fragmentation of the cellular DNA and, thereby, leading to apoptosis. The lack of change in mitochondrial transmembrane potential combined with their activation of caspase 3/7 and 8 suggest that apoptosis induced by tambjamines I and J is triggered by an extrinsic pathway. Accordingly, compounds **2**–**6** and **8**−**11** can be regarded as potentially useful lead compounds for drug development and further investigations into their *in vitro* activities are warranted. Investigations of this type will be facilitated by the ready capacity to prepare tambjamine analogues through the Schiff-base condensation of a wide range of amines with aldehydes such as **17** and **54**.

CHAPTER TWO

THE TOTAL SYNTHESES OF CERTAIN MONOMERIC EPOXYQUINOL NATURAL PRODUCTS

2.1 INTRODUCTION TO THE MONOMERIC EPOXYQUINOL NATURAL PRODUCTS

(+)-Bromoxone (**55**), (+)-bromoxone acetate (**106**), (+)-epiepoformin (**108**), (−)-harveynone (**148**) and (−)-tricholomenyn A (**174**) are prominent and representative members of a significant and growing class of epoxyquinol-based natural products.[77,78] (See Figure 2-1).

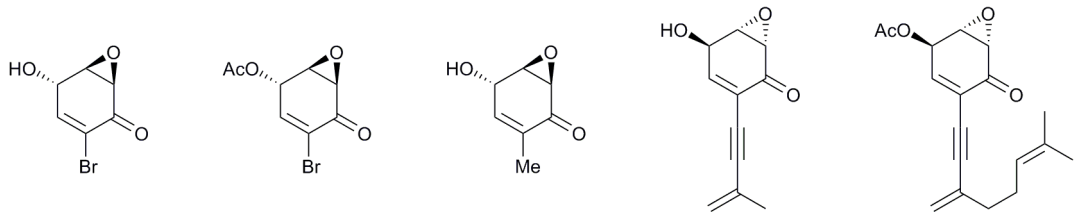
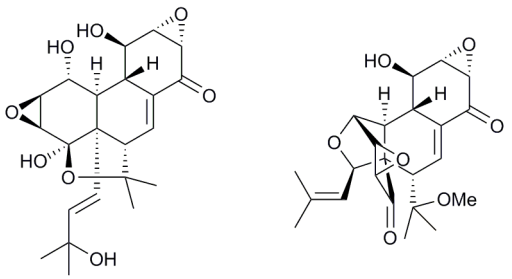


Figure 2-1: *Representative epoxyquinol-type natural products*

The densely funtionalized cyclohexene frameworks associated with these compounds together with their diverse range of biological properties have prompted a great deal of effort to establish economical syntheses of them.[77-102] Much of this effort has relied upon the acquisition of protected forms of bromoxone or its iodo analogue that are then subjected to Heck, Sonogashira, Suzuki, or Stille reactions with the relevant cross-coupling partner.[86,89,90,93-96,99] The required haloxone derivative is often generated from benzoquinone or protected forms thereof[79-81,85,88-90,94,97,98,101] while enzymatically-mediated desymmetrization or resolution processes[80,81,83,85,90,94,96,98,99] have been used to obtain the required substrates in enantiomerically pure form. As a consequence, somewhat lengthy reaction sequences can be involved. Accordingly, the research described here was focussed upon the development of abbreviated syntheses of the epoxyquinols as well as the certain complex epoxyquinol-derived natural products. Ultimately, these efforts were fruitful.[103,104] A description of the range of epoxyquinol natural products and their total syntheses are presented immediately below while syntheses of

the structurally more complex dimeric systems (+)-panepophenanthrin (**192**) and (+)-hexacyclinol (**233**), shown in Figure 2-2, are discussed in Chapter Three.



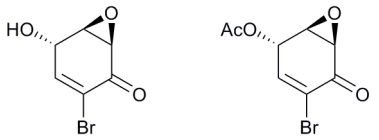
192 233

Figure 2-2: *(+)-Panepophenanthrin (**192**) and (+)-hexacyclinol (**233**)*

2.2 (+)-BROMOXONE

2.2.1 Isolation and characterization of (+)-bromoxone

(+)-Bromoxone (**55**) and its *O*-acetyl derivative (**106**) (Figure 2-3) were both isolated from a marine acorn worm and the latter was shown to display anti-tumour properties.[105] The first of these two compounds, **55**, was characterized spectroscopically while the absolute configuration of naturally-derived acetate **106** was determined by single-crystal X-ray analysis. Acetylation of the naturally-derived alcohol **55** afforded a material identical to ester **106**, as judged by ^{1}H NMR spectroscopic and tlc analyses, thus supporting the structure proposed by Higa *et al*. for the latter compound.[105]



55 106

Figure 2-3: *(+)-Bromoxone (**55**) and its* O*-acetyl derivative, (+)-bromoxone acetate (**106**)*

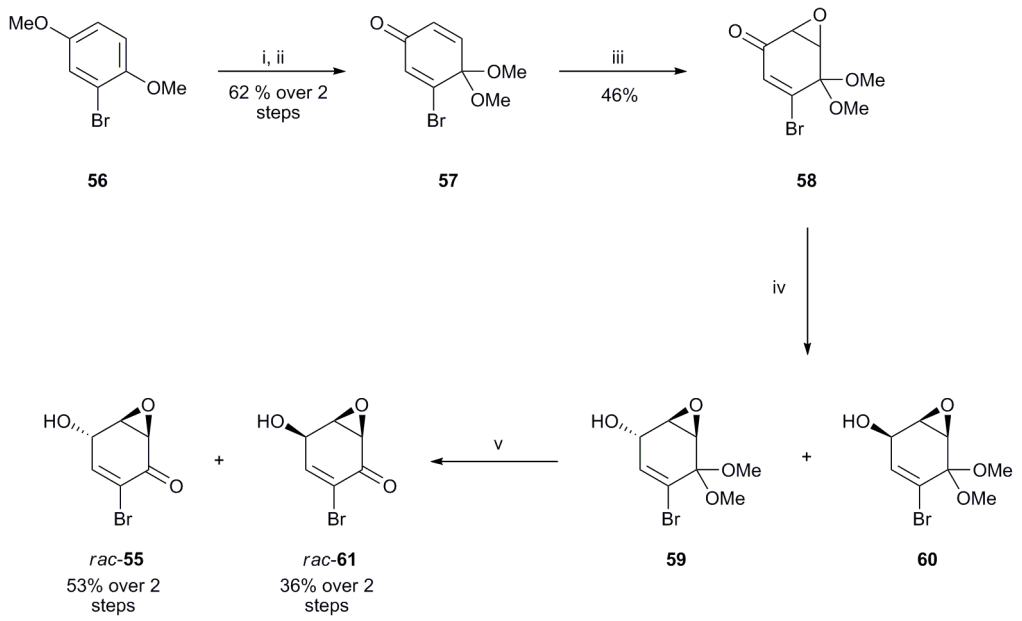
2.2.2 Previous studies on the synthesis of bromoxone

Due to the importance of bromoxone as a synthon *en route* to more elaborate epoxyquinol compounds, it has been the subject of a number of total syntheses.[79-83] A discussion of each of these is presented immediately below.

Taylor's total synthesis of (±)-bromoxone (1994)

The first synthesis of bromoxone was described by Taylor *et al.*[79] His approach, leading to the racemate, is shown in Scheme 2-1 and started with the anodic oxidation of 2-bromo-1,4-

dimethoxybenzene (**56**). Regioselective epoxidation of the ensuing quinone monoketal **57** followed by stereoselective reduction of the ketone moiety within product **58** delivered a 2:1 mixture of the diastereoisomeric ketals **59** and **60**. Exposure of these compounds to Montmorillonite K10 clay led to ketones *rac*-**55** and *rac*-**61**, respectively, and these could be readily separated by conventional chromatographic methods. In this way, pure samples of (±)-bromoxone (*rac*-**55**) and (±)-epibromoxone (*rac*-**61**) were obtained with the former product predominating.

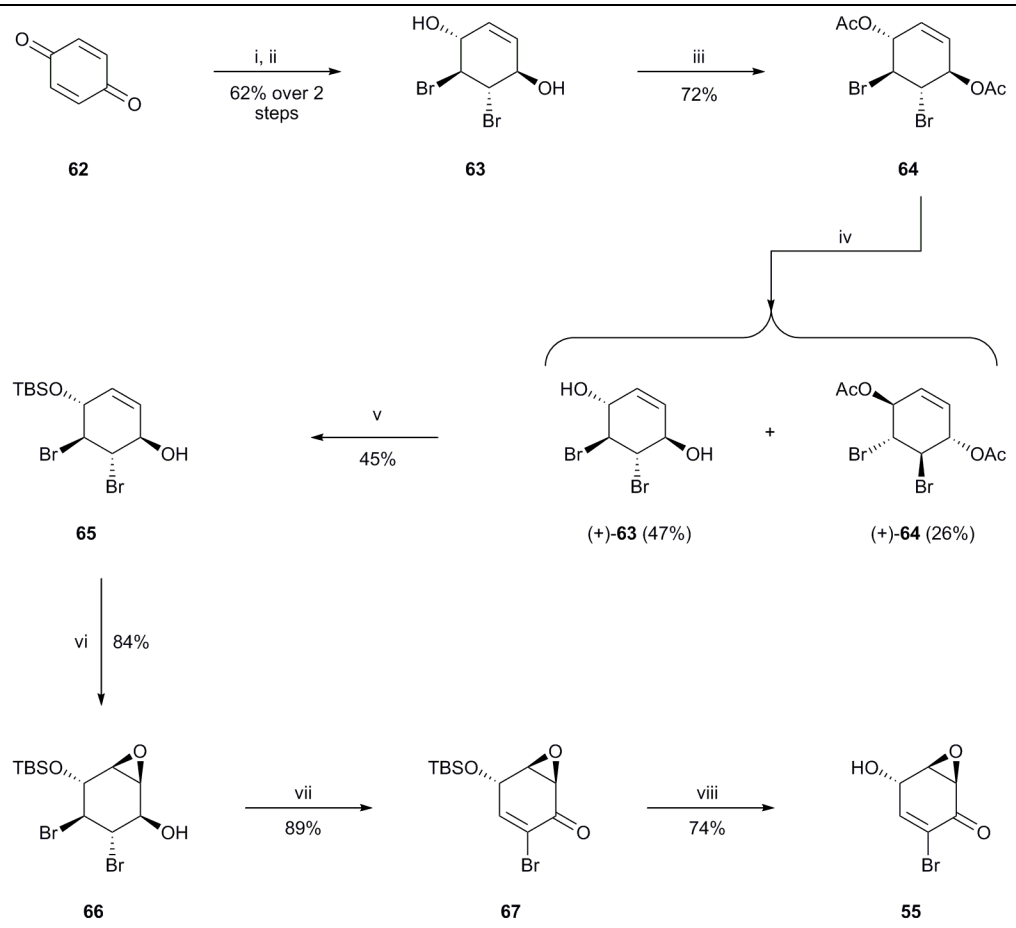


Scheme 2-1: *Reagents* (i) anodic oxidation; (ii) AcOH, acetone; (iii) *t*-BuOOH, KH; (iv) DIBAL-H; (v) K-10.

Johnson's total synthesis of (+)-bromoxone (1995)

In 1995 Johnson *et al*.[80] developed a total synthesis of (+)-bromoxone using the route shown in Scheme 2-2. Thus, *p*-benzoquinone (**62**) was dibrominated with molecular bromine and the ketone moieties within the ensuing product were reduced with sodium borohydride to afford the *bis*-allylic diol **63**. Acylation of both hydroxyl groups within compound **63** using acetic anhydride afforded diester **64**. Chemoenzymatic resolution of the racemic *bis*-acetates **64** using *Pseudomonas cepacia* lipase (Amano PS-30) effected selective hydrolysis of the levorotatory component and provided the corresponding diol [(+)-**63**], from which (+)-bromoxone was subsequently prepared. Thus, mono-protection of diol (+)-**63** as the silyl ether **65** followed by electrophilic epoxidation of the latter compound with peracetic acid gave epoxy alcohol **66** in a diastereoselective manner. Treatment of this compound with the Jones' reagent effected oxidation of the alcohol moiety and thereby facilitated elimination of the elements of HBr to afford enone **67**. Finally, treatment of compound **67** with hexafluorosilicic acid provided (+)-bromoxone (**55**) in 74% yield.

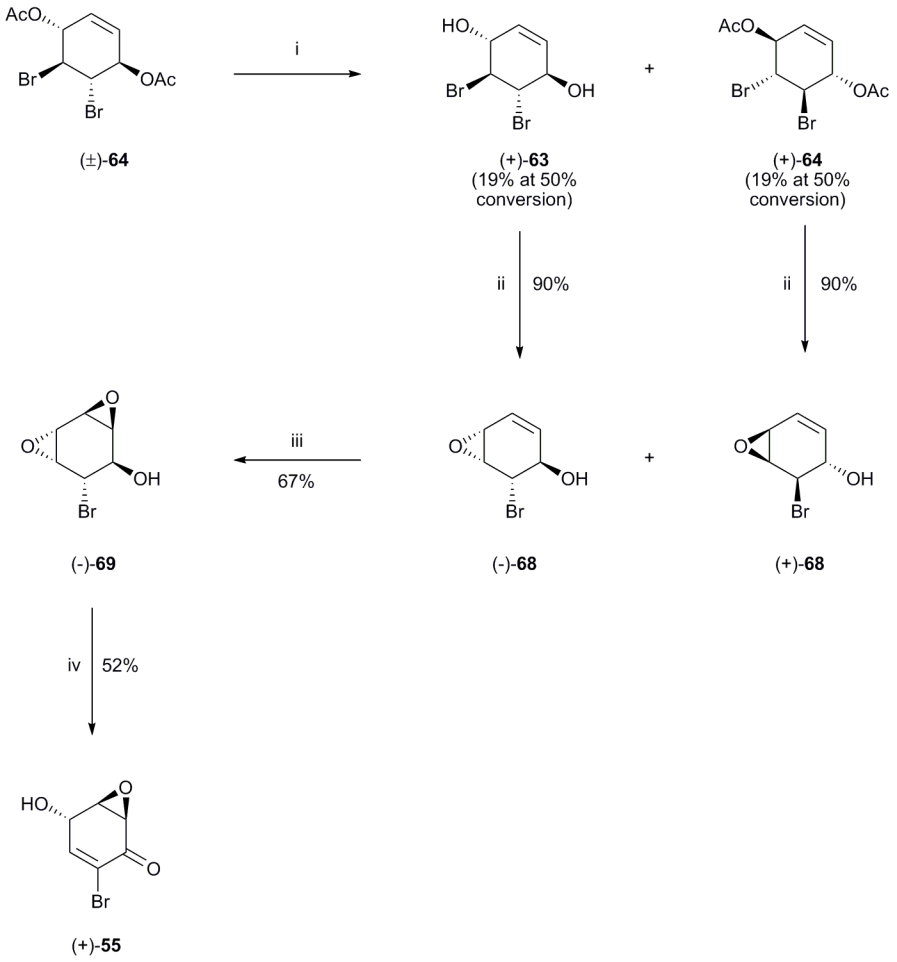
Although not depicted, (−)-bromoxone was readily obtained from diester (+)-**64**. This enantiomer, though resistant to the action of Amano PS-30, underwent cleavage of both acetates with titanium(IV) isopropoxide in isopropanol and it was shown that by adhereing to a procedure parallel to that described for the synthesis of (+)-bromoxone, the optical antipode, (−)-bromoxone (*ent*-**55**) could be obtained.



Scheme 2-2: *Reagents* (i) Br_2; (ii) $NaBH_4$; (iii) Ac_2O, Et_3N, DMAP; (iv) Amano PS-30, pH = 8 phosphate buffer; (v) TBSOTf, Et_3N; (vi) CF_3CO_3H, Na_2HPO_4; (vii) $CrO_3(py)_2$; (viii) H_2SiF_6.

Altenbach's total synthesis of (+)-bromoxone (2000)

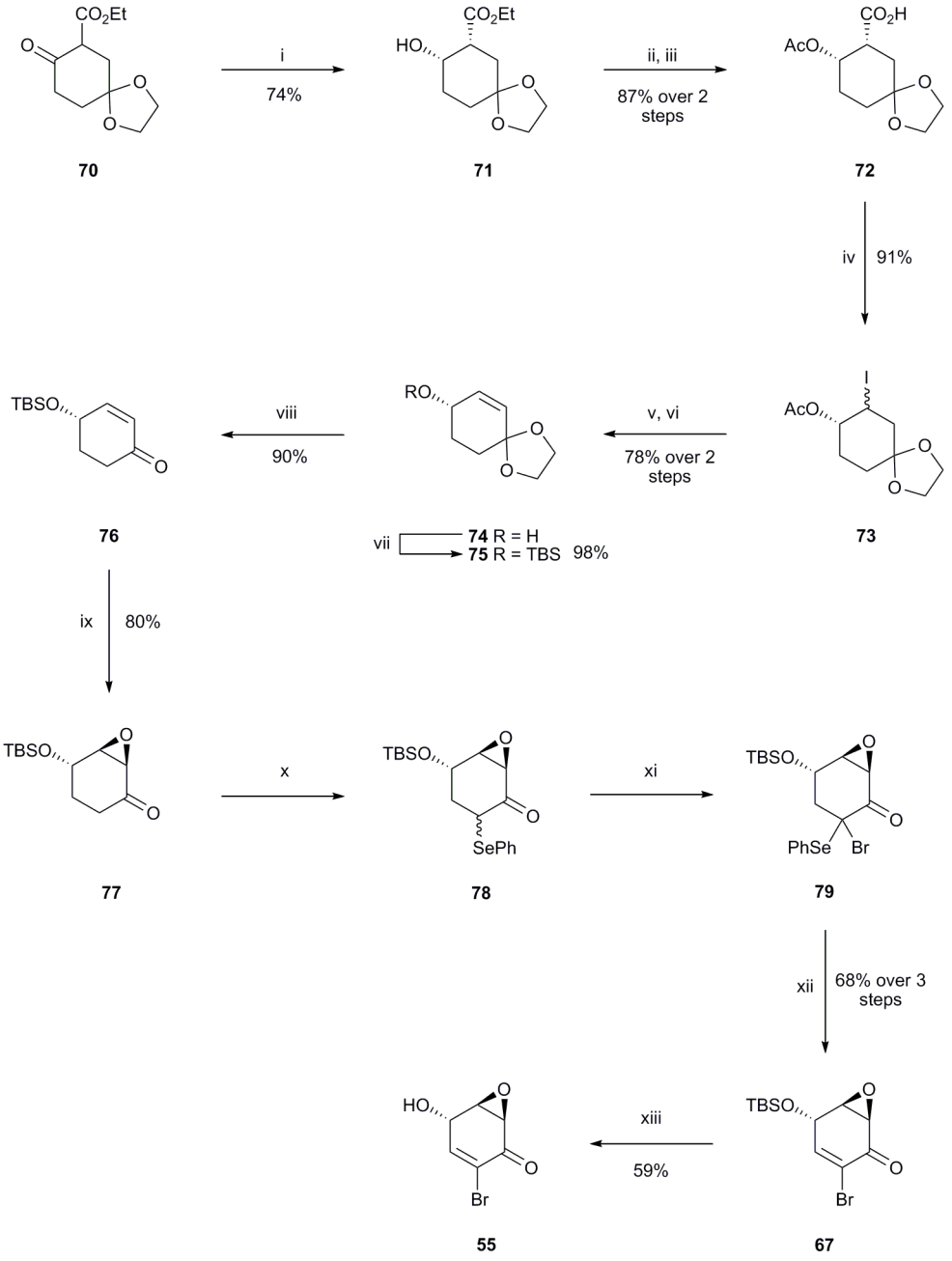
(+)-Bromoxone was synthesized by Altenbach *et al*. using the protocol described in Scheme 2-3.[81] These researchers used pig pancreas lipase (PPL) to effect resolution of the enantiomeric forms of diester (±)-**64**, a process analogous to that previously described by Johnson *et al*.[80] (+)-Bromoxone was obtained from the liberated diol [(+)-**63**] *via* two rounds of epoxidation followed by a Dess-Martin periodinane-mediated oxidation of the ensuing alcohol (−)-**69**. Concomitant and regioselective epoxide-ring-opening within the product ketone then delivered the target compound (+)-**55**.



Scheme 2-3: *Reagents* (i) PPL, buffer pH = 7; (ii) LiOH; (iii) *m*-CPBA; (iv) DMP.

Kitahara's total synthesis of (+)-bromoxone (2003)

The Kitahara[83] synthesis of (+)-bromoxone started with a chiral substrate, namely ester **71** which was obtained *via* the highly enantioselective reduction of β-ketoester **70** using baker's yeast (Scheme 2-4). The ethyl ester moiety within product **71** was then hydrolyzed and the hydroxyl group acylated under standard conditions to afford the acetoxy acid **72**. This material underwent the Suárez-modified Hunsdiecker reaction to afford iodide **73**. Elimination of the elements of hydroiodic acid from within compound **73** could be effected with DBU and provided an intermediate allylic alcohol, the hydroxyl group of which was protected to afford silyl ether **75**. Hydrolysis of the ketal moiety within the latter material was effected with PPTS in aqueous methanol and nucleophilic epoxidation of the resulting enone delivered epoxy-ketone **77**. An intermediate silyl-enol-ether was generated from this material and this was treated with phenylselenium chloride to give α-selenoketone **78**. Bromination of this compound under basic conditions then afforded compound **79**. Oxidative elimination of the phenylselenyl moiety within compound **79**, followed by treatment of the product enone with hydrofluoric acid then yielded (+)-bromoxone in 13% yield over 13 steps.

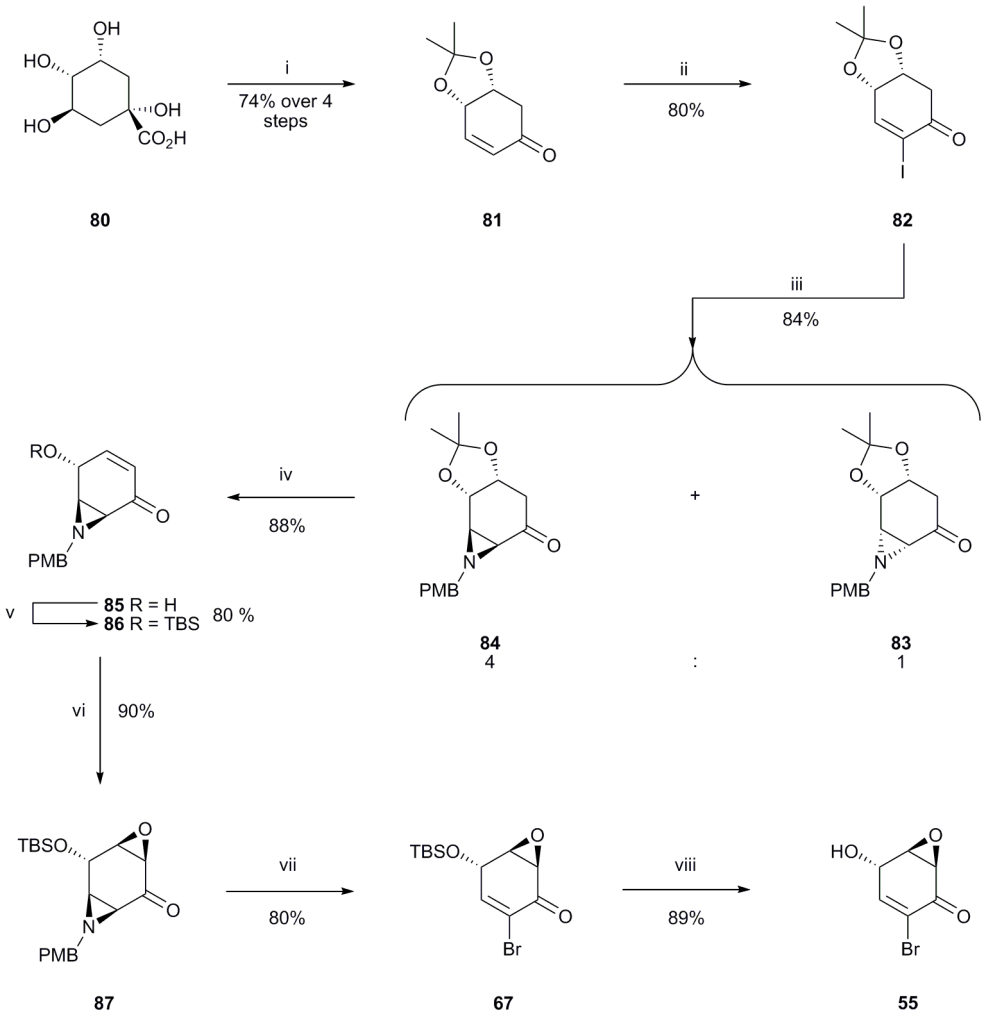


Scheme 2-4: *Reagents* (i) dry baker's yeast; (ii) LiOH; (iii) Ac_2O, pyridine; (iv) IBDA, I_2; (v) DBU; (vi) K_2CO_3; (vii) TBSCl, imidazole; (viii) PPTS, aq. Me_2CO; (ix) H_2O_2, TritonB; (x) LiHMDS, TMSCl, PhSeCl; (xi) DBU, Br_2; (xii) 35% H_2O_2, $NaHCO_3$; (xiii) 40% HF.

Maycock's total synthesis of (+)-bromoxone (2003)

The first synthesis of (±)-bromoxone that did not rely upon an enzymatic resolution was described in 2003 by Maycock *et al*.[82] Thus, as depicted in Scheme 2-5, the α,β-unsaturated cyclohexenone **81**, which was obtained in four steps from the abundant chiron quinic acid (**80**), served as the starting material and was iodinated at the α-position using the Johnson procedure.[106] Gabriel-Cromwell aziridination of the product α-iodoenone with 4-methoxybenzylamine provided a mixture of the expected diastereoisomeric products **83** and **84** with the latter predominating. Isomer **84** was treated with sodium hydroxide and through loss of

the elements of acetone, an alkene was thereby installed on that side of the six-membered ring opposite the aziridine moiety itself. The hydroxyl group of product **85** was converted into the corresponding TBS ether **86** which was subject to selective nucleophilic epoxidation. The facial selectivity of this reaction was directed by the bulky silyl ether residue. The aziridine ring of product **87** was selectively reacted with dilute HBr to give α-bromoenone **67**. Treatment of silyl ether **67** with HF in acetonitrile then gave (+)-bromoxone in 18% overall yield and seven steps from quinic acid (**80**).



Scheme 2-5: *Reagents* (i) As described by Maycock *et al.*[106] (ii) I_2, DMAP, pyridine; (iii), 4-methoxybenzylamine, Cs_2CO_3, 1,10-phenanthroline; (iv) NaOH; (v) TBSCl, DMAP, $(i\text{-Pr})_2NEt_3$; (vi) H_2O_2, Triton B; (vii) HBr; (viii) HF.

2.3 TOTAL SYNTHESIS OF (−)-BROMOXONE AND ITS CHLORO- AND IODO-ANALOGUES

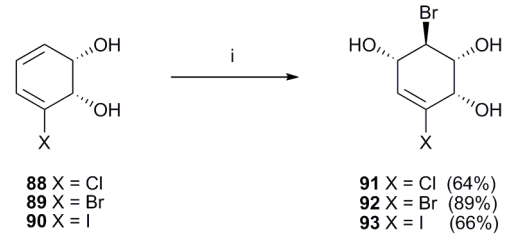
2.3.1 Synthetic strategy associated with the present synthesis of the tambjamines

The development of a rapid synthesis of bromoxone was considered a worthy objective as it was anticipated that by doing so, a range of related compounds would also become more accessible. In fact, bromoxone and its iodo-congener or protected forms thereof have served as intermediates *en route* to several more elaborate targets. The descriptions of previous syntheses of enantiomerically pure bromoxone given above reveal the lengthy nature of the reaction sequences required to obtain this important synthetic intermediate. To overcome such difficulties, a facile and chemoenzymatic route to several versatile epoxyquinol synthons was developed.[104] Thus, these densely functionalized synthons were obtained in just four steps from certain *cis*-1,2-dihydrocatechols that are themselves readily obtained in large quantity and enantiomerically pure form *via* the enzymatic dihydroxylation of the corresponding halobenzenes. The value of these synthons is highlighted, as detailed in the following sections, through their application to abbreviated total syntheses of (−)-bromoxone (*ent*-**55**), its *O*-acetyl congener (*ent*-**106**), (+)-epiepoformin (*ent*-**108**), (−)-harveynone (**148**) and (−)-tricholomenyn A (**174**). Details of these are presented below. Related syntheses of (+)-panepophenanthrin (**192**), and (+)-hexacyclinol (**233**), structurally more complex natural products arising from the Diels-Alder dimerization of epoxyquinol monomers, are discussed separately in Chapter Three.

2.3.2 Regio- and diastereo-selective bromohydrin-forming reactions

The dihydrocatechols **88**-**90**, readily obtained from the whole-cell biotransformation of the corresponding halobenzene,[107-109] were employed as the starting materials in syntheses of (−)-bromoxone and its chloro- and iodo-congeners. The reaction sequences started with treatment of the diols with NBS in aqueous THF and thereby affording bromohydrins **91**-**93** in a completely regio- and diastereo-selective manner. This selectivity is the result of certain electronic and steric effects. Thus, formation of an intermediate bromonium cation takes place first of all and this forms at the more electron-rich of the two double bonds within the starting material. Inductive withdrawal of electron density by the appended halogen sufficiently deactivated the associated alkene that reaction at this alternate position was not observed. Steric effects exerted by the hydroxyl groups directed electrophilic attack by bromine from the less hindered β-face. Subsequent nucleophilic opening of the resulting bromonium species by water takes place exclusively at the allylic position and thereby providing the illustrated bromohydrins **91**-**93** in yields that ranged from 64 to 89% (Scheme 2-6). The outcomes of these

bromohydrination reactions are in accord with the results of a study by Seonane *et al*. on the halohydroxylation of a range of protected dihydrocatechols.[110]



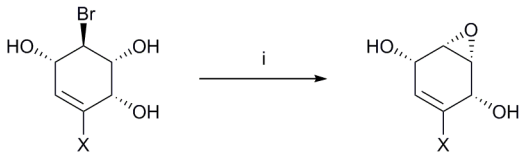
88 X = Cl
89 X = Br
90 X = I

91 X = Cl (64%)
92 X = Br (89%)
93 X = I (66%)

Scheme 2-6: *Reagents and conditions* (i) NBS, H_2O, THF, 20 °C, 1h.

2.3.3 Regioselective epoxide-forming reactions

Treatment of the bromohydrins **91-93** with sodium methoxide in THF resulted in selective epoxide ring formation and generation of compounds **94-96** in yields ranging from 52 to 79%. (See Scheme 2-7).



91 X = Cl
92 X = Br
93 X = I

94 X = Cl (52%)
95 X = Br (56%)
96 X = I (79%)

Scheme 2-7: *Reagents and conditions* (i) NaOMe, THF, 20 °C, 1h.

The ^{13}C NMR spectrum recorded on bromoalkene **95** is shown in Figure 2-4 and reveals signals due to two olefinic carbons at δ 130.4 and 124.0. The hydroxyl groups gave rise to a broad absorption band at 3229 cm^{-1} in the IR spectrum while in the EI mass spectrum of the mono-brominated diol **95** displays molecular ions of approximately equal intensity at *m*/*z* 208 and 206. Such an isotopic distribution is expected for a mono-brominated compound. An accurate mass measurement carried out on the former species established the molecular formula of the compound is $C_6H_7{}^{79}BrO_3$ as expected for the assigned structure **95**.

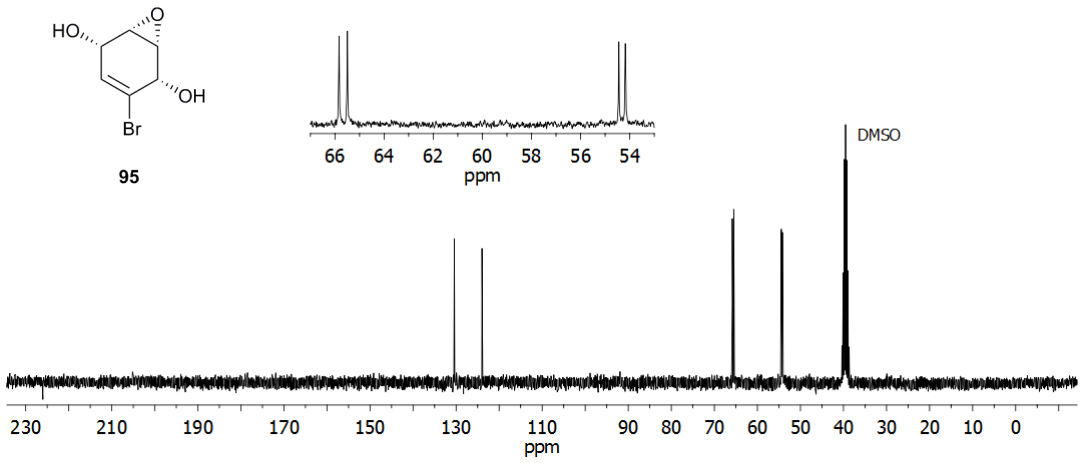


Figure 2-4: *75 MHz ^{13}C NMR spectrum of epoxide **95** (recorded in d_6-DMSO). Inset: An expansion of a region from the spectrum.*

The structures of the epoxides **94-96** were confirmed by single-crystal X-ray analyses carried out on each of them. An ORTEP derived from the single-crystal X-ray analysis of epoxide **95** is shown in Figure 2-5. The anisotropic displacement ellipsoids show 30% probability levels. Full X-ray structure reports are provided in the appendix.

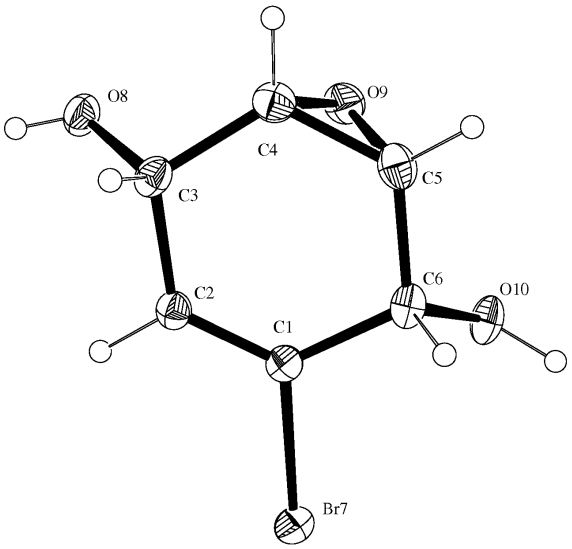
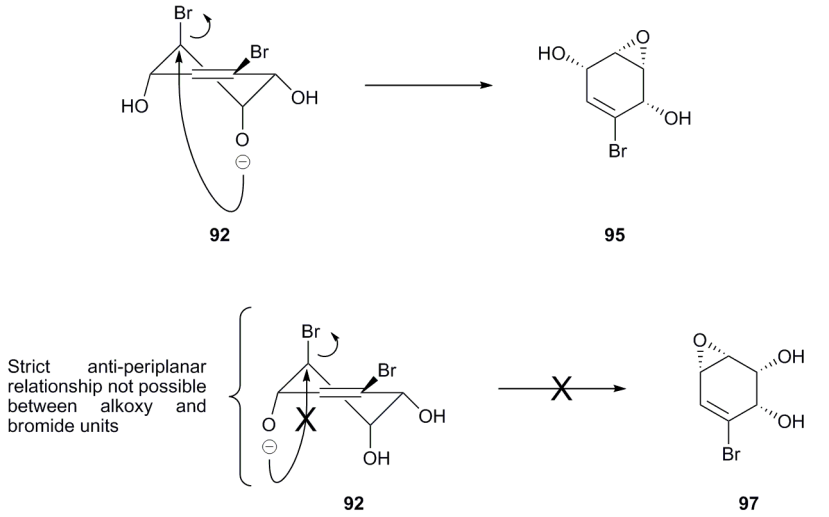


Figure 2-5 *ORTEP derived from single-crystal X-ray analysis of **95**.*

The specific rotation ($[\alpha]_D$) of diol **95** was +16.6 (*c* 5.0, THF). A comparison of this value with those recorded for compounds **94** {$[\alpha]_D$ +5.6 (*c* 5.0, THF)} and **96** {$[\alpha]_D$ +26.4 (*c* 5.0, THF)}, indicated that the magnitude of specific rotation increased with the increasing size of the appended halogen atom.

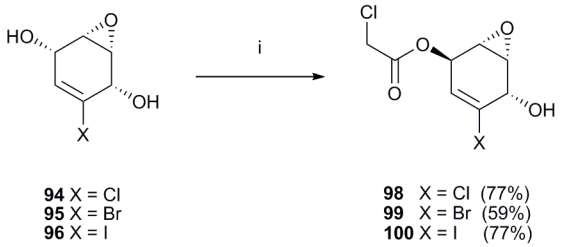
The high level of selectivity associated with the formation of epoxides **94**-**96** from bromohydrins **91**-**93** is somewhat surprising at first glance given that in each of the precursors **91**-**93** the departing bromine is *trans*-related to two adjacent hydroxyl groups and thus there is, in principle at least, the possibility of forming an isomeric epoxide. It is believed that stereoelectronic constraints inhibited the oxygen at the allylic position from participating in the alternate epoxide-forming reaction, as shown in Scheme 2-8.



Scheme 2-8: *The formation of alkenyl epoxides such as **97** were not observed during the base induced epoxidation reactions of bromohydrins **91**-**93**.*

2.3.4 Regioselective Mitsunobu reactions

Mitsunobu reaction of *cis*-diols **94**-**96**, using chloroacetic acid as the nucleophile, took place selectively at the allylic hydroxyl moiety remote from the halogen atom, presumably because it is less sterically encumbered (Scheme 2-9). Initially, di*iso*propyl azodicarboxylate (DIAD) was used in this reaction however the byproduct arising from it, namely di*iso*propyl hydrazinodicarboxylate, often co-eluted with the product during chromatographic purification on silica gel. Fortunately, di*tert*butyl azodicarboxylate proved to be a suitable alternative since the corresponding by-product, di*tert*butyl hydrazinodicarboxylate, has a higher mobility on silica gel during chromatography and was, therefore, readily separated from the product by such means.



94 X = Cl
95 X = Br
96 X = I

98 X = Cl (77%)
99 X = Br (59%)
100 X = I (77%)

Scheme 2-9: *Reagents and conditions* (i) $(t\text{-BuOCON})_2$, PPh_3, $ClCH_2CO_2H$, THF, 20 °C, 0.25 h.

The ^{1}H NMR spectrum of product **99** (Figure 2-6) displays one-proton multiplets at δ 3.66 and 3.50 that are assigned to the protons associated with the epoxide ring. A two-proton singlet appearing at δ 4.10 is assigned to the CH_2 unit of the chloroacetyl residue. Unsurprisingly, the spectrum recorded on the corresponding chloro- and iodo-analogues, **98** and **100** respectively, are very similar. The ester moieties installed during the Mitsunobu reaction gave rise to strong C=O stretching band at 1750 cm^{-1} in the IR spectrum of each of the compounds **98**-**100**.

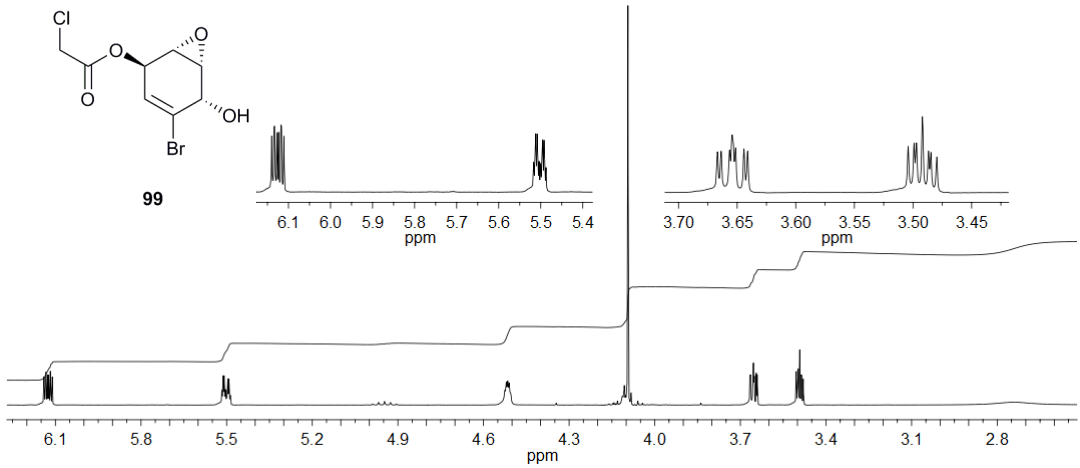
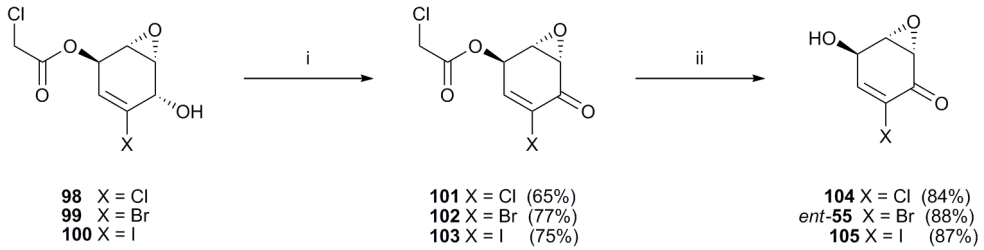


Figure 2-6: *300 MHz ^{1}H NMR spectrum of ester **99** (recorded in $CDCl_3$)*
Insets: Expansions of two regions from the spectrum.

2.3.5 Alcohol oxidation and ester cleavage

Pyridinium dichromate in dichloromethane containing a small amount of acetic acid was used to oxidize the hydroxyl group in each of the allylic alcohols **98**-**100** to the corresponding enones **101**-**103** respectively (Scheme 2-10). Acetic acid was added in order to accelerate the oxidation reaction.[111] The oxidation products were isolated in yields ranging from 65 to 77%. During attempts to remove the α-chloroacetyl protecting group from these enones they were exposed to methanolic potassium carbonate. Unfortunately, these conditions caused rapid decomposition of the substrate and/or product. Even the use of relatively mild bases such as pyridine and sodium bicarbonate did not alleviate this problem, perhaps due to the facile nature of base-induced aromatization processes. However, smooth cleavage of the chloroacetyl esters could be effected

using zinc (II) acetate dihydrate in methanol. Under such conditions the chloroacetates could be cleaved and (−)-bromoxone (*ent*-**55**) and its chloro- and iodo-congeners (**104** and **105**, respectively) were thereby obtained in yields ranging from 84 to 88%.



Scheme 2-10: *Reagents and conditions* (i) PDC, AcOH, DCM, 20 °C, 1 h; (ii) $Zn(OAc)_2.2H_2O$, MeOH, 20 °C, 1 h.

The ^{1}H NMR spectrum of (−)-bromoxone (Figure 2-7) exhibited all of the expected proton signals except for that derived from the hydroxyl proton that had presumably undergone deuterium for proton exchange with the solvent. The specific rotation of this material {$[\alpha]_D$ −187.1 (*c* 1.75, acetone)} was of similar magnitude but opposite sign to that reported for (+)-bromoxone {$[\alpha]_D$ +205.7 (*c* 0.32, acetone)}.[106] The remaining spectral data derived from this material were identical, in all respects, with those reported in the literature for its optical antipode.[79-83,105]

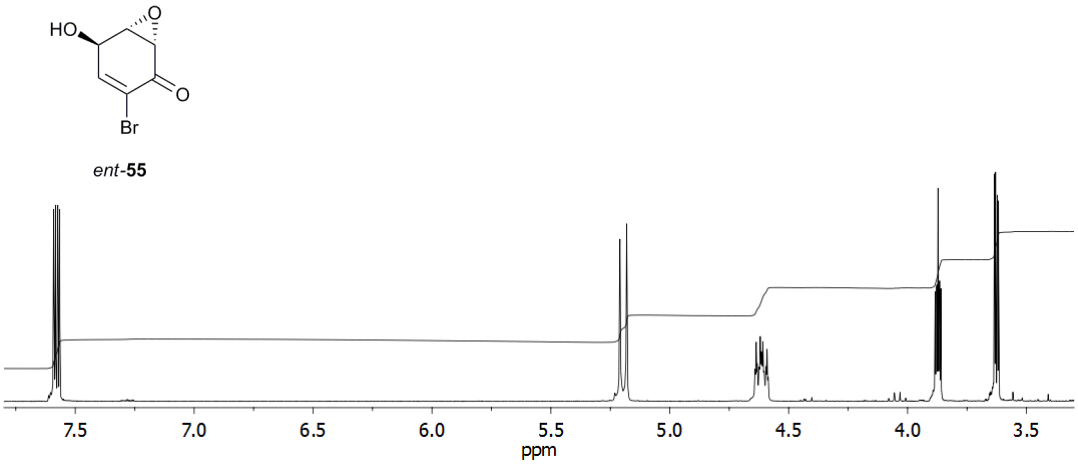


Figure 2-7: *300 MHz ^{1}H NMR spectrum of (−)-bromoxone (recorded in d_6-acetone).*

The data presented Table 3-1 reveal that the present work provides the shortest total synthesis of enantiomerically pure bromoxone. The overall yield for the synthesis described here is unsurpassed and the average yield per reaction step is 81%. Furthermore, as the starting material for the synthesis, *cis*-dihydrocatechol **89**, is derived from abundant bromobenzene, the entire carbon framework of the final product is derived from this commodity chemical. Conveniently, every step of the synthesis of (−)-bromoxone disclosed in this work is conducted at standard temperatures and pressures.

Table 2-1: *A comparison of the key features associated with the seven reported syntheses of bromoxone*

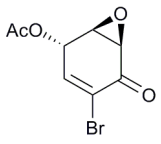
Lead author	Publication date	Longest linear sequence	Overall yield (%)	Modification produced
Taylor[79]	1994	five steps	15	(±)
Johnson[80]	1995	eight steps	5	(+)
Altenbach[81]	2000	six steps	14	(+)
Kitahara[83]	2003	thirteen steps	13	(+)
Maycock[82]	2003	eight steps	18	(+)
Present work[104]	2009	five steps	34	(−)
Schapiro[112]	2010	twelve steps	4	(+)

The publication of the work described here was followed by a report by Schapiro *et al*. on the total synthesis of (+)-bromoxone from diol **89** in 12 steps and an overall yield of 4%.[112] Deprotection of a certain intermediate on this route also provided diol **95**, in five steps and 53% yield from the starting diol **89**, and thereby constituted a formal total synthesis of (−)-bromoxone.

2.4 (+)-BROMOXONE ACETATE

2.4.1 Isolation and characterization of (+)-bromoxone acetate

(+)-Bromoxone acetate (**106**) and its *O*-deacetyl congener (+)-bromoxone were both isolated from a marine acorn worm (Phylum Himichordata, Order Enteropneusta) and the structure of the former was confirmed by single-crystal X-ray analysis.[105] Compound **106** was also shown to be active against P388 leukemia cells (IC_{50} = 10 ng mL^{-1}), a property not displayed by the corresponding alcohol.



106

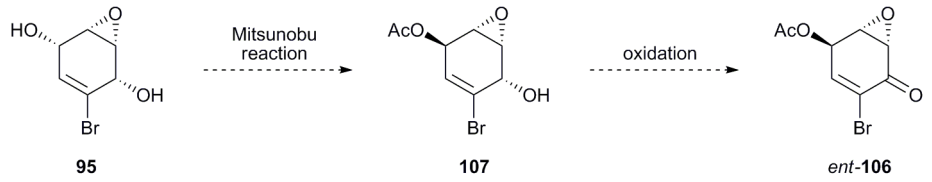
Figure 2-8: *(+)-Bromoxone acetate.*

2.5 SYNTHETIC STRATEGY ASSOCIATED WITH THE PRESENT SYNTHESIS OF (−)-BROMOXONE ACETATE

Although bromoxone (**55**) has been the subject of a number of total syntheses, its *O*-acetyl congener (**106**) has not been. In consideration of the successful application of the Mitsunobu reaction *en route* to (−)-bromoxone, as described in the previous section, it was anticipated that similar methodology would provide an especially concise route to (−)-bromoxone acetate (*ent*-**106**). Details of the successful outcome of such an approach are presented below.

2.5.1 Proposed synthetic route to (−)-bromoxone acetate

The previously described and readily available bromoepoxide **95** was selected as the synthon from which (−)-bromoxone acetate would be synthesized. It was anticipated that subjection of this diol to a Mitsunobu reaction, using acetic acid as the nucleophile, would install the acetate residue with the desired stereochemistry (Scheme 2-11). Oxidation of the ensuing alcohol **107**, in a manner identical to that applied to alcohol **99** in the abovementioned synthesis of (−)-bromoxone, should then deliver α,β-unsaturated ketone *ent*-**106** and complete the synthesis of (−)-bromoxone acetate in just four steps from the commercially available *cis*-dihydrocatechol **89**. The successful implementation of these ideas are presented in the following sections.



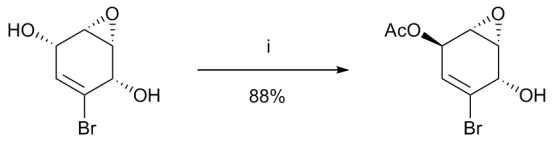
95 **107** ***ent*-106**

Scheme 2-11: *Proposed synthetic route to (−)-bromoxone acetate*

2.6 TOTAL SYNTHESIS OF (−)-BROMOXONE ACETATE

2.6.1 The Mitsunobu reaction

In keeping with the synthetic plan presented immediately above, bromodiol **95** was submitted to a Mitsunobu reaction with acetic acid (Scheme 2-12) using conditions analogous to those employed earlier in the synthesis of (−)-bromoxone. Once more, the Mitsunobu reaction took place selectively at the less-hindered allylic alcohol and provided the anticipated acetate **107** in 88% yield as a white, crystalline solid.



95 **107**

Scheme 2-12: *Reagents and conditions* (i) (*t*-BuOCON)$_2$, PPh$_3$, AcOH, THF, 20 °C, 1 h.

The ^{1}H NMR spectrum of acetate **107** (Figure 2-9) is reminiscent of that obtained from α-chloroacetate **99**. Thus, the signal arising from the olefinic proton appeared at δ 6.11 while the hydroxyl group proton was observed as a doublet (J = 9.9 Hz) at δ 2.80. The acetate group carbons gave rise to signals in the ^{13}C NMR spectrum at δ 169.9 and δ 20.7 whilst the other signals in the same spectrum showed only small differences with respect to chemical shifts observed for the analogous carbons of the α-chloroacetate **99**. An hydroxyl group absorption

band was observed at 3457 cm^{-1} in the IR spectrum along with bands at 1737 and 1231 cm^{-1} that are attributed to C=O and C−O stretching, respectively.

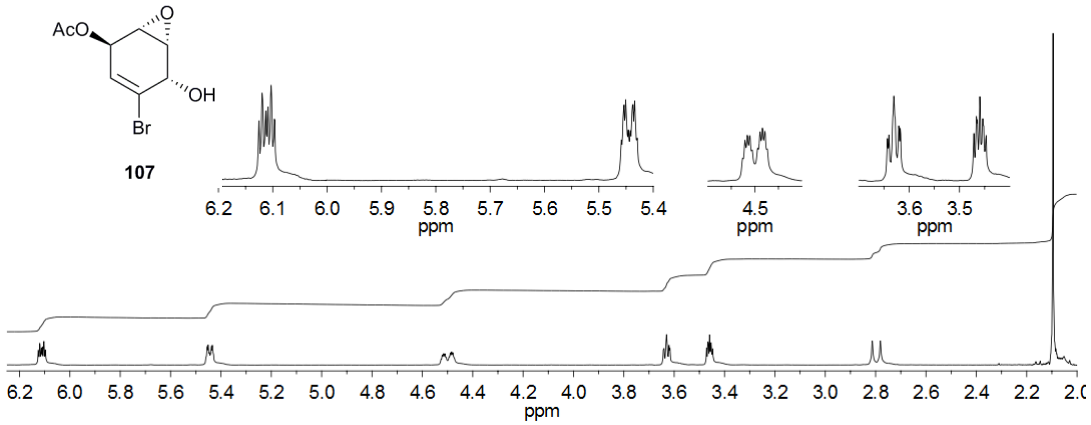
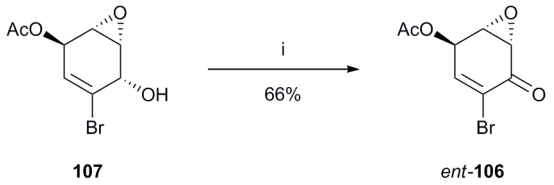


Figure 2-9: *300 MHz ^{1}H NMR spectrum of alcohol **107** (recorded in $CDCl_3$).*
Insets: Expansions of three regions from the spectrum.

The EI mass spectrum of acetate **107** shows molecular ions at *m*/*z* 248 and 250 and these were of approximately equal intensity. Such an isotopic distribution is indicative of a mono-brominated compound. Accurate mass measurement on the lighter of these established that it was of the expected composition, *viz*. $C_8H_9{}^{79}BrO_4$.

2.6.2 Alcohol oxidation

Oxidation of alcohol **107** was best effected with pyridinium dichromate (PDC) in the presence of a small amount of acetic acid and using dichloromethane as solvent (Scheme 2-13).[111] The (−)-bromoxone acetate (*ent*-**106**) was thereby obtained in 51% yield.



Scheme 2-13: *Reagents and conditions* (i) PDC, AcOH, DCM, 20 °C, 1 h.

The specific rotation of *ent*-**106** {$[\alpha]_D$ −278.2 (*c* 2.55, chloroform)} was of similar magnitude but opposite sign to that reported for (+)-bromoxone acetate {$[\alpha]_D$ +265 (*c* 0.12, chloroform)}. The associated ^{1}H spectrum is shown in Figure 2-10 and matches that reported for the natural product.[105]

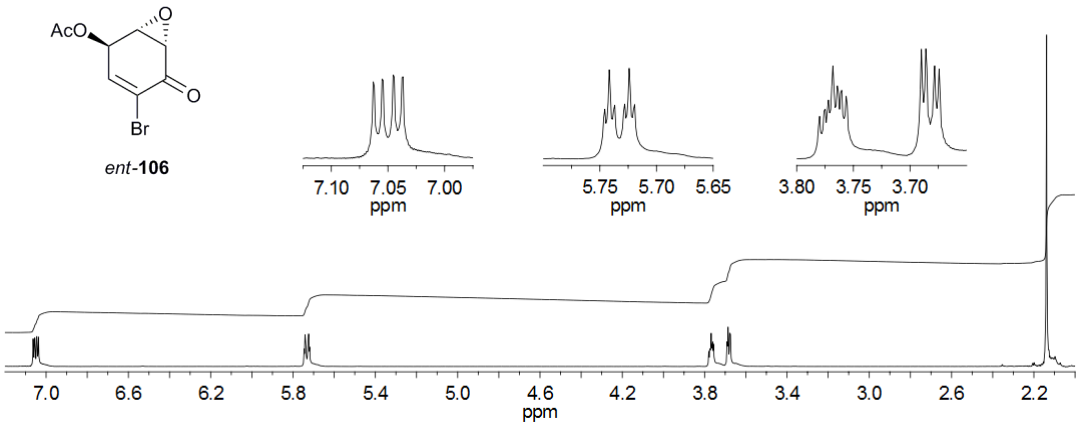


Figure 2-10: *300 MHz ^{1}H NMR spectrum of (−)-bromoxone acetate (recorded in $CDCl_3$). Insets: Expansions of three regions from the spectrum.*

A comparison of the ^{1}H NMR data recorded for naturally- and synthetically-derived bromoxone acetate (**106** and *ent*-**106**, respectively) is shown in Table 2-2. The observed chemical shifts differ by no more than 0.02 ppm while the *J* values, where direct comparisons are possible, vary by less than 0.2 Hz.

Table 2-2: *Comparison of the ^{1}H NMR data recorded on naturally- and synthetically-derived bromoxone acetate (**106** and* ent-***106**, respectively)*

^{1}H NMR Data Natural **106** (δ_H) (300 MHz, $CDCl_3$)[105]	^{1}H NMR Data Synthetic *ent*-**106** (δ_H) (300 MHz, $CDCl_3$)
7.04 (dd, *J* = 5.3 and 2.3 Hz, 1H)	7.05 (dd, *J* = 5.4 and 2.4 Hz, 1H)
5.73 (dt, *J* = 5.3 and 1.3 Hz, 1H)	5.73 (ddd, *J* = 5.4, 1.2 and 1.2 Hz, 1H)
3.75 (ddd, *J* = 3.4, 2.3 and 1.3 Hz, 1H)	3.77 (m, 1H)
3.68 (dd, *J* = 3.4 and 1.2 Hz, 1H)	3.68 (dd, *J* = 3.6 and 1.2 Hz, 1H)
2.13 (s, 3H)	2.14 (s, 3H)

While the remaining spectral data derived from the oxidation product *ent*-**106** depicted in Scheme 2-12 were identical, in all respects, with those reported in the literature for its optical antipode, final confirmation of the structure of compound *ent*-**106** came from a single-crystal X-ray analysis. The ORTEP obtained from this analysis is shown in Figure 2-11. The anisotropic displacement ellipsoids show 30% probability levels. The full X-ray structure report is provided in the appendix.

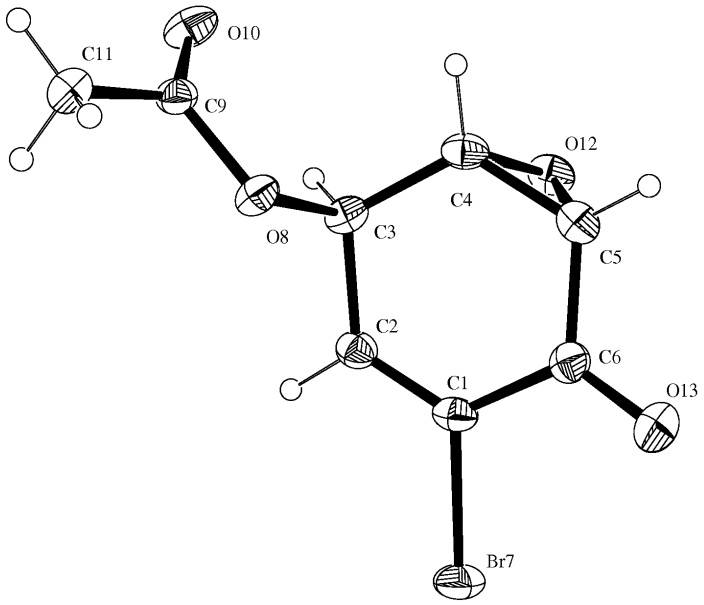
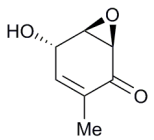


Figure 2-11: *ORTEP derived from single-crystal X-ray analysis of (−)-bromoxone acetate.*

2.7 (+)-EPIEPOFORMIN

2.7.1 Isolation and characterization of (+)-epiepoformin

The title compound (**108**) was isolated by Nagasawa *et al*.[113] from a culture of an unidentified fungus that was, itself, obtained from a diseased leaf of the crape-myrtle tree (*Lagerstroemia indica* L.). The compound was found to exhibit phytotoxic properties, including a capacity to inhibit the germination of lettuce seeds, *Lactuca sativa* L. The basic structure of (+)-epiepoformin was established by conventional means while circular dichroism (CD) techniques were used to determine its absolute stereochemistry.



108

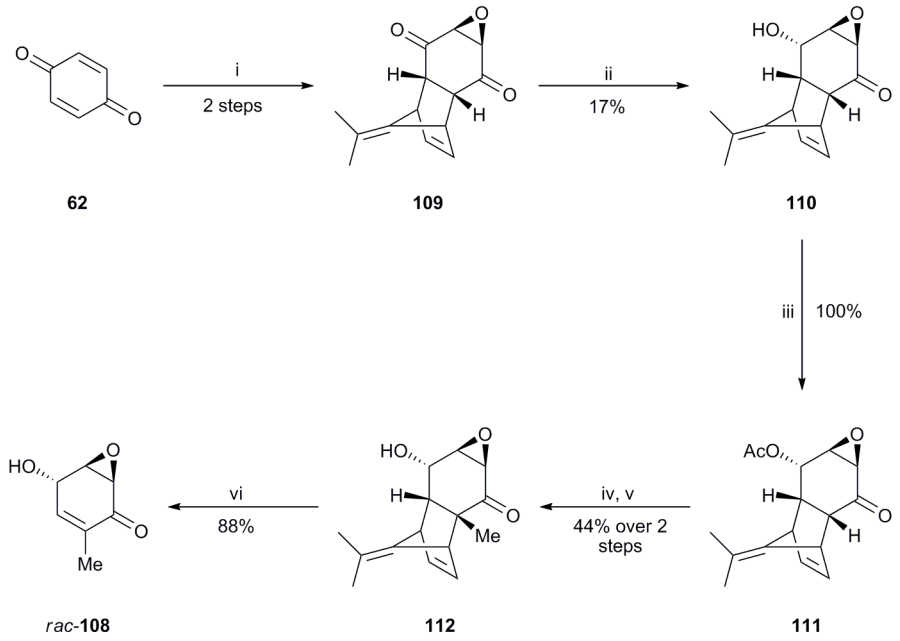
Figure 2-12: *(+)-Epiepoformin*

2.7.2 Previous studies on the synthesis of epiepoformin

Epiepoformin has been the subject of six prior total syntheses.[83-88] The first of these involved the synthesis of the racemate from *p*-benzoquinone. All subsequent syntheses have led to the naturally-occurring enantiomeric form of the natural product.

Ichihara's total synthesis of (±)-epiepoformin (1982)

The first synthesis of epiepoformin, reported by Ichihara *et al*.[84] in 1982, provided the racemic modification of the natural product. This approach, which is shown in Scheme 2-14, used *p*-benzoquinone (**62**) as the starting material. This was engaged in a Diels-Alder reaction with 6,6-dimethylfulvene and the conjugated double bond within the adduct was then epoxidized in a diastereoselective fashion to afford compound **109**.[114] Sodium borohydride was then used to selectively reduce one of the two ketone moieties within this compound and thereby produce an epimeric mixture of alcohols, the illustrated *trans*-epoxy alcohol **110** being obtained in 17% yield and the epimeric *cis*-epoxy alcohol (not shown) being obtained in 19% yield. Compound **110** was protected as the corresponding acetate, **111**, under standard conditions and this was then *C*-alkylated using standard techniques to give, after acetate group cleavage, alcohol **112**. Heating this last compound in ethyl acetate at 140 °C (sealed tube) effected a retro-Diels-Alder reaction and thereby afforded (±)-epiepoformin (*rac*-**108**) in 88% yield.

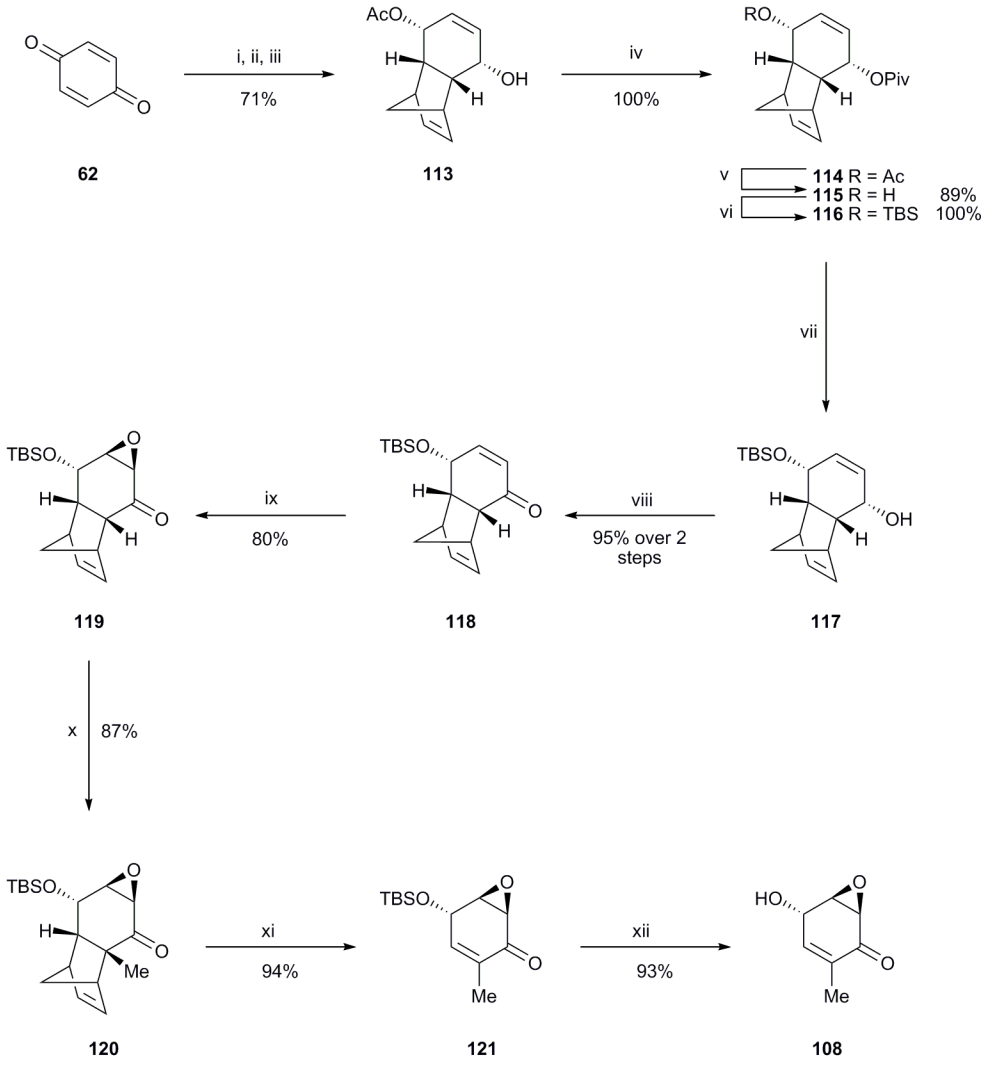


Scheme 2-14: *Reagents* (i) As described by Ichihara *et al*.;[114] (ii) $NaBH_4$; (iii) Ac_2O, pyridine; (iv) KO*t*-Bu, MeI; (v) KOH (10%); (vi) heated at 140 °C for 20 min.

Ogasawara's total synthesis of (+)-epiepoformin (1995)

Ogasawara *et al*.[85] described the first enantiocontrolled synthesis of (+)-epiepoformin in 1995 (Scheme 2-15). The sequence commenced with the optically pure tricyclic allylic alcohol **113** that had been obtained through the lipase-mediated desymmetrization of a *meso*-diol precursor derived from *p*-benzoquinone (**62**).[24,115,116] The absolute configuration of acetate **113** was established using chemical correlation studies.[115] Following protection of alcohol **113** as the corresponding pivalic ester, **114**, the acetyl residue within the latter compound was exchanged, *via* two standard steps, for a *tert*-butyldimethylsilyl group. The ensuing pivalic ester **116** was

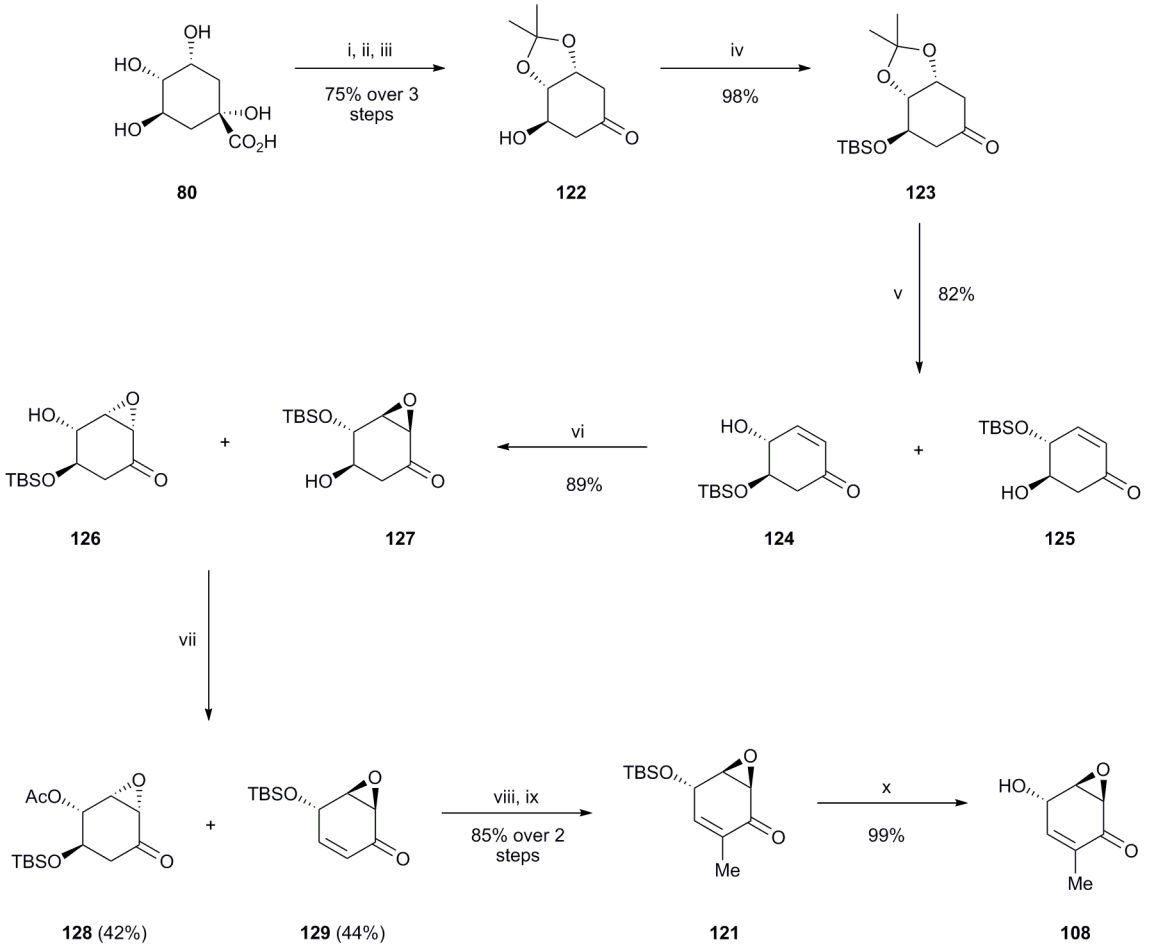
then treated with methyl lithium in order to cleave the pivaloyl protecting group and thereby affording compound **117**. PDC-mediated oxidation of allylic alcohol **117** produced an intermediate enone from which epoxy-ketone **119** was obtained *via* nucleophilic epoxidation with alkaline hydrogen peroxide. The epoxidation reaction proceeded in a facially-selective manner. An axial methyl group, delivered to the *exo*-face of the enolate derived from ketone **119**, was installed adjacent to the ketone using potassium hydride and methyl iodide. The product of this alkylation reaction, epoxy-ketone **120**, was then heated in diphenyl ether in order to effect a retro-Diels-Alder reaction and thereby restore the alkene moiety present in the natural product. This process afforded silyl ether **121** in a high yield and, following HF-mediated desilylation of this compound, a 12-step synthesis of (+)-epiepoformin was completed. This proceeded in 37% overall yield from *p*-benzoquinone (**62**). The acquisition of this material served to confirm the absolute configuration originally assigned to the natural product.



Scheme 2-15: *Reagents* (i) heated at 40 °C for 3 h; (ii) $(i\text{-Pr})_2AlH$; (iii) vinyl acetate, Amano PS-30 lipase; (iv) pivalic anhydride, NEt_3, DMAP; (v) K_2CO_3, MeOH; (vi) TBS-OTf, Et_3N; (vii) MeLi; (viii) PDC; (ix) 30% H_2O_2, Triton B; (x) KH, MeI; (xi) reflux with diphenyl ether; (xii) 45% HF-MeCN.

Maycock's total synthesis of (+)-epiepoformin (2000)

Maycock *et al*.[86] have developed a series of relatively efficient syntheses of natural products closely related to (+)-epiepoformin (**108**). These were readily modified to give target **108** that was obtained *via* the route shown in Scheme 2-16. Thus, β-hydroxyketone **122**, which was produced in three steps from (−)-quinic acid (**80**), was *O*-silylated with *tert*-butyldimethylsilyl chloride[106] and the ensuing ether **123** underwent base-induced elimination of the elements of acetone to give α,β-unsaturated enone **124**. This was accompanied by the *trans*-*O*-silylation product such that a 1:1 mixture of isomers **124** and **125** was obtained. Recycling of the chromatographically separated isomer **125** delivered more of the desired isomer **124** under the same basic conditions. Though these regioisomers could be separated, it was considered more expedient to engage the mixture in nucleophilic epoxidation and by such means the corresponding mixture of epoxyketones **126** and **127** were obtained. These compounds were acetylated and the 5-acetoxy intermediate derived from compound **127** underwent elimination (presumably *via* an E1cb pathway) of the elements of acetic acid to give enone **129**. The latter compound was easily separated from the residual 4-acetoxy byproduct using chromatographic methods and then carried forward in the synthesis. Thus, α-iodination of enone **129** with molecular iodine and Stille cross-coupling of the ensuing organoiodide with tetramethyltin afforded the required α-methylated enone **121**. Finally, fluoride-mediated desilylation of silyl ether **121** delivered (+)-epiepoformin (**108**) that was obtained in ten steps and 20% overall yield from (−)-quinic acid. This synthesis represents a more direct approach to compound **108** than an earlier variant described by Maycock that also started from (−)-quinic acid.[117]

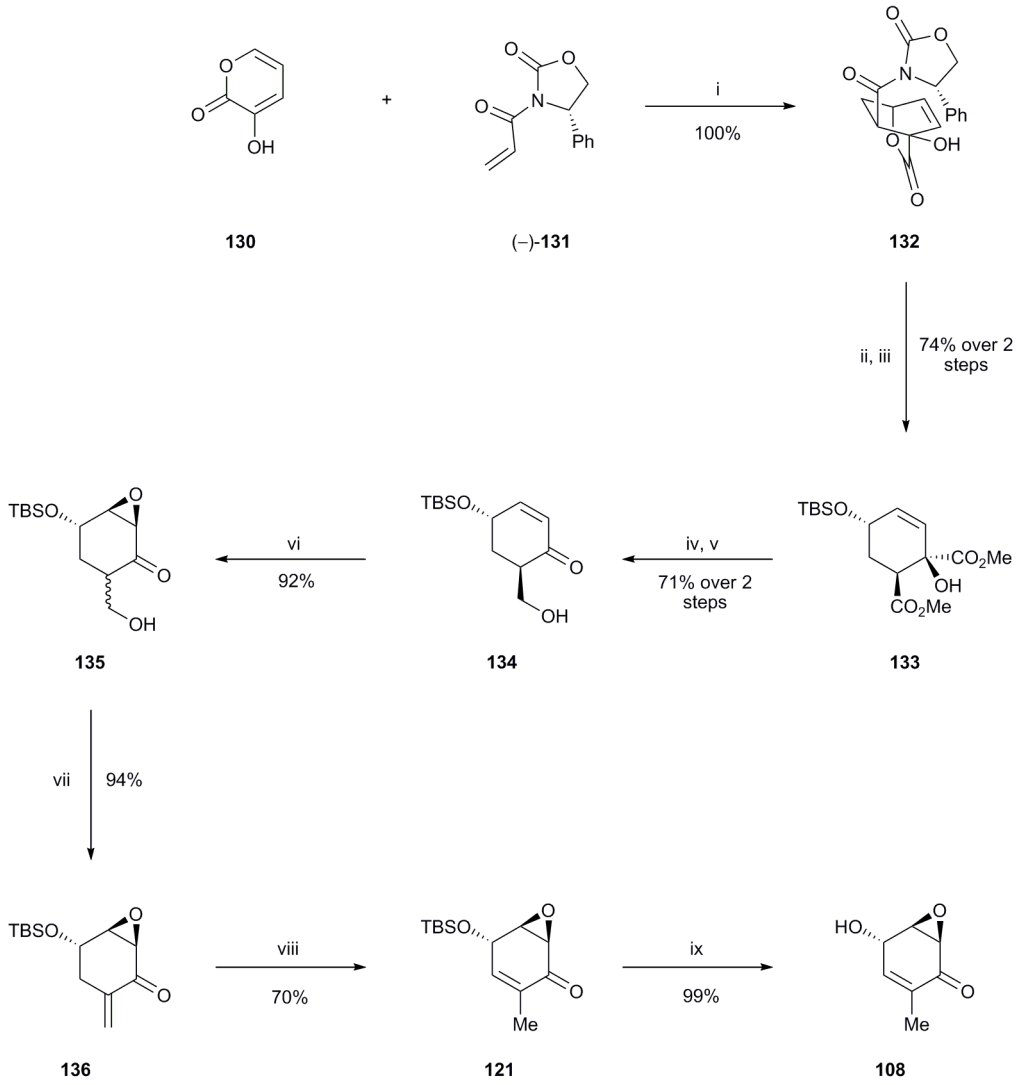


Scheme 2-16: *Reagents* (i) Acetone, dry HCl; (ii) Ac_2O, pyridine; (iii) (a) $LiAlH_4$, (b) $NaIO_4$; (iv) TBS-Cl, imidazole; (v) 0.5 N NaOH; (vi) 30% H_2O_2, Triton B; (vii) Ac_2O, $(i\text{-Pr})_2NEt$, DMAP; (viii) I_2, DMAP, pyridine; (ix) Me_4Sn, $AsPh_3$, $Pd_2(dba)_3{\cdot}CHCl_3$, CuI; (x) HF (40% in H_2O).

Okamura's total synthesis of (+)-epiepoformin (2001)

The key step in the Okamura synthesis of (+)-epiepoformin (Scheme 2-17) was the cinchonine-catalyzed Diels-Alder reaction of 3-hydroxy-2-pyrone (**130**) with acrylate (+)-**131**.[87,118] While the resulting adduct **132** was employed in the depicted synthesis of (+)-epiepoformin, it is noteworthy that its optical antipode is accessible from pyrone **130** by utilizing a different chiral auxiliary and catalyst system. Exposure of compound **132** to sodium methoxide effected displacement of the chiral oxazolone residue and concomitant opening of the bridging lactone linkage, such that diester **133** was obtained. Reduction of both methyl ester moieties within compound **133** was effected with lithium aluminium hydride and the resulting triol was exposed to sodium periodate. Under these conditions, the vicinal diol moiety was oxidatively cleaved and ketone **134** thus obtained. α-Face-directed nucleophilic epoxidation of compound **134** was effected with basic hydrogen peroxide and afforded epoxyketone **135**. Tosylation of the β-hydroxyl moiety within compound **135** and its subsequent elimination with triethylamine then afforded enone **136**. Palladium on carbon was used to effect the isomerization of this alkene to congener **121**, from which (+)-epiepoformin was readily obtained *via* desilylation with HF. This

process thus afforded the natural product in nine steps and in an overall yield of 31% from 3-hydroxy-2-pyrone (**130**).

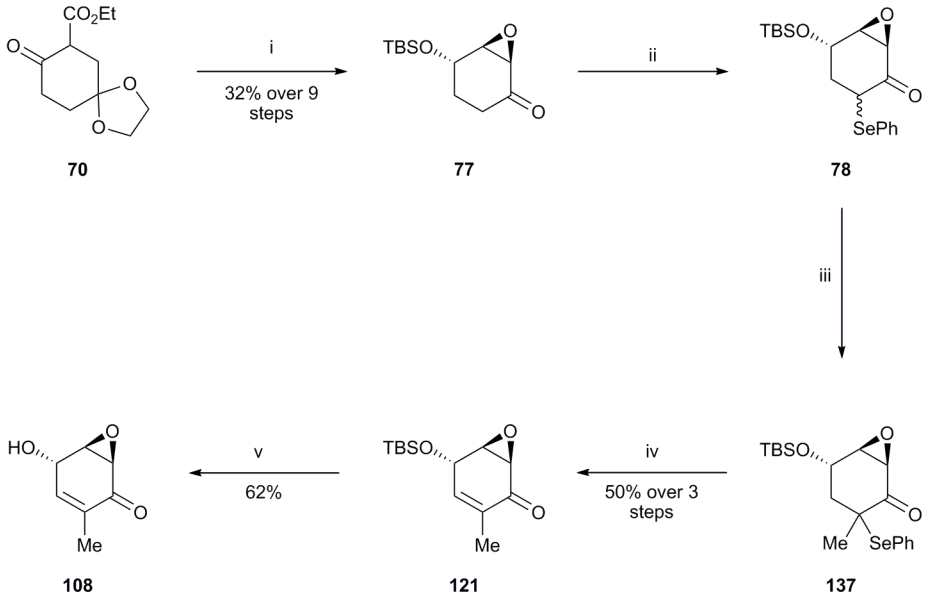


Scheme 2-17: *Reagents* (i) cinchonine; (ii) TBS-Cl, imidazole; (iii) NaOMe; (iv) $LiAlH_4$; (v) $NaIO_4$; (vi) 30% aq. H_2O_2, Triton B; (vii) *p*-TsCl, Et_3N, DMAP; (viii) Pd/C; (ix) HF.

Kitahara's total synthesis of (+)-epiepoformin (2003)

Kitahara *et al.*[83] synthesized (+)-epiepoformin from intermediate epoxyketone **77**, a compound that was used in their earlier synthesis of (+)-bromoxone (described in Section 2.2.2). Thus, α-selenation of compound **77** with phenylselenium chloride proceeded *via* the silyl-enol-ether intermediate and delivered selenide **78** as a mixture of diastereomers. The enolate derived from compound **78** was then quenched with methyl iodide to yield compound **137** which was also obtained as a mixture of diastereomers. The selenide moiety within compound **137** was then oxidized with hydrogen peroxide and the resulting selenoxide eliminated thermally to provide enone **121** from which (+)-epiepoformin was obtained in 62% yield *via* a fluoride-induced

deprotection step. In this way, the natural product was obtained in a 10% overall yield and in 13 steps from β-keto-ester **70**.

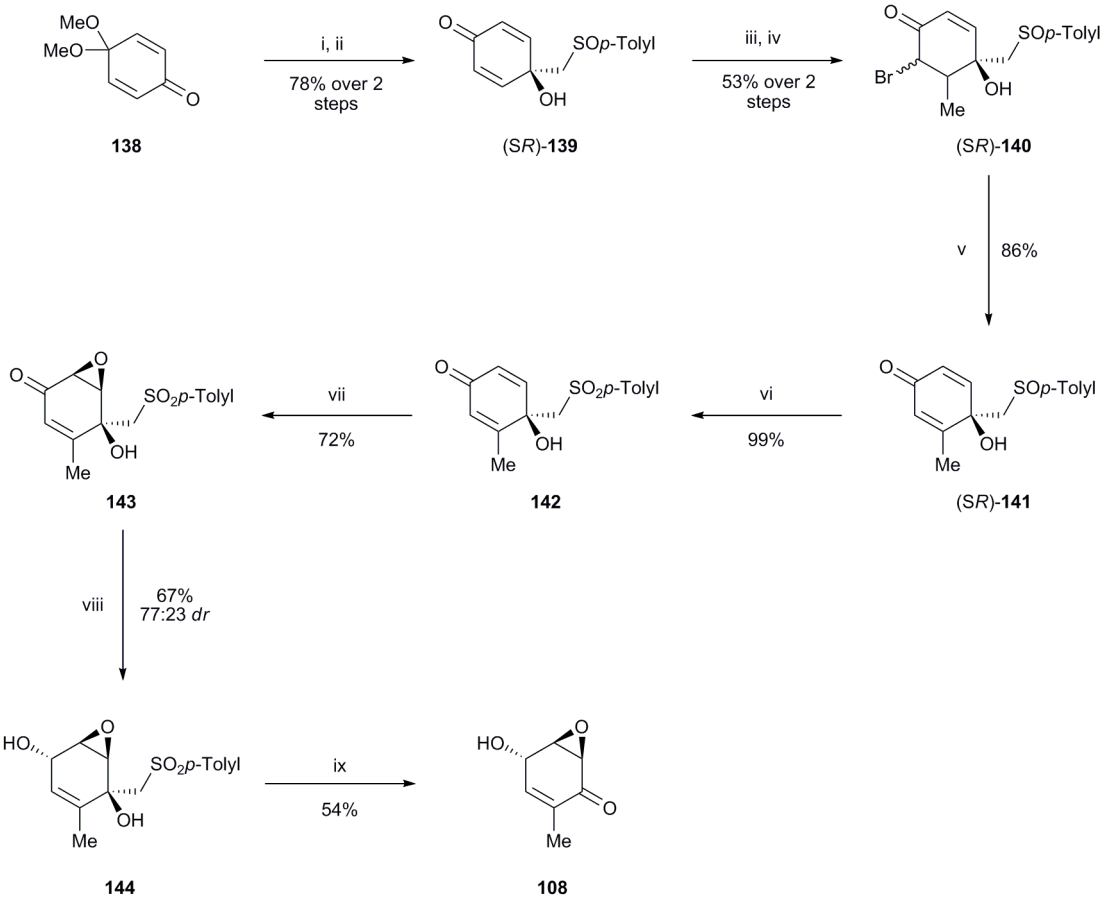


70 **77** **78** **108** **121** **137**

Scheme 2-18: *Reagents* (i) As described by Kitahara and Tachihara;[83] (ii) LiHMDS, TMS-Cl, PhSeCl; (iii) NaH, MeI; (iv) 35% aq. H_2O_2, $NaHCO_3$; (v) 40% aq. HF.

Carreno's total synthesis of (+)-epiepoformin (2005)

The total synthesis of (+)-epiepoformin developed by Carreno *et al*.[88] utilized the addition of the lithium anion of (S,*S*)-methyl *p*-tolylsulfoxide to the carbonyl of *p*-benzoquinone dimethyl acetal (**138**). The intermediate acetal was then cleaved with oxalic acid to give the dienone (S,*R*)-**139**. Using Me_3Al, a methyl group was delivered in a diastereo- and chemo-selective fashion to the β-position of one of the enone moieties associated with compound (S,*R*)-**139**. The resulting aluminium enolate was then *C*-brominated with NBS. The product of this process, the diastereoisomeric mixture of bromo-ketones (S,*R*)-**140**, was treated with Li_2CO_3/LiCl mixtures and thus effecting loss of the elements of HBr to regenerate the 5,6-olefin present in (S,*R*)-**141**. The sulfone moiety within compound (S,*R*)-**141** was then oxidized to the corresponding sulfonate in anticipation of its eventual removal. In the meantime, the less-substituted alkene of compound **142** was selectively oxidized with *tert*-butyl hydroperoxide and Triton B in THF to afford epoxyketone **143**. The ketone moiety within this last compound was then stereoselectively reduced with di*iso*butyl aluminium hydride to afford alcohol **144**. The elimination of the elements of methyl *p*-tolylsulfone from compound **144** was effected with cesium carbonate in methanol and produced (+)-epiepoformin (**108**) in 54% yield. While the yield encountered in this elimination step could be improved if the primary hydroxyl group is first protected as the corresponding *tert*-butyldimethylsilyl ether, the losses associated with the requisite protection and deprotection steps required for the alternate preparation of this compound make this other route less efficient.



Scheme 2-19: *Reagents* (i) (*SS*)-methyl *p*-tolylsulfoxide, LDA; (ii) oxalic acid; (iii) Me_3Al; (iv) NBS; (v) Li_2CO_3, LiBr; (vi) *m*-CPBA; (vii) *t*-BuOOH, Triton B; (viii) $(i\text{-Bu})_2AlH$; (ix) Cs_2CO_3.

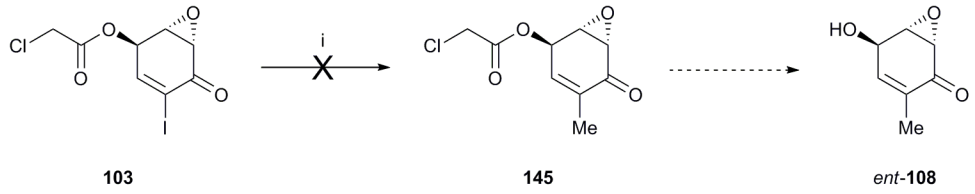
2.8 SYNTHETIC STRATEGY ASSOCIATED WITH THE PRESENT SYNTHESIS OF (−)-EPIEPOFORMIN

Although epiepoformin has been the subject of six total syntheses (the first afforded a racemic sample of the natural product and the rest provided (+)-epiepoformin), the generation of (−)-epiepoformin has not been reported previously. In light of the rapid synthesis of (−)-bromoxone described in the preceeding section, it was expected that exploitation of a similar methodology would lead to a concise total synthesis of (−)-epiepoformin (*ent*-**108**), the biological evaluation of which would be of some interest.

2.8.1 Proposed synthetic route to (−)-epiepoformin

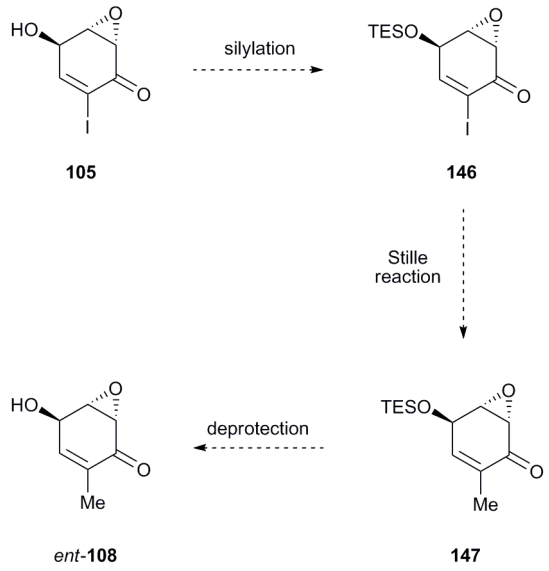
Iodide **103**, the synthesis of which was described in Section 2.3.5, was selected as an attractive precursor to (−)-epiepoformin because it bears the same oxygenation pattern as the target molecule and the halogen atom is positioned appropriately for the intended cross-coupling reaction with tetramethyltin. In the interests of maximizing the reactivity of the substrate, it was the iodinated (rather than the chlorinated or brominated) congener that was to be utilized in

palladium-catalyzed cross-coupling reactions described here. In the event, however, iodide **103** failed to deliver the desired cross-coupled product (Scheme 2-20).



Scheme 2-20: *Reagents and conditions* (i) Me_4Sn, Pd[0], Ph_3As, CuI, THF, 100 °C, 1 h.

Fortunately, successful Stille cross-coupling reactions with an *O*-silyl compound analogous to ester **103** have been described previously.[86] On this basis, silyl ether **146** was considered an appropriate substrate for a Stille cross-coupling reaction with tetramethyltin (Scheme 2-21). In this case, desilylation of the cross-coupling product **147** should deliver (−)-epiepoformin. The successful implementation of this approach is described in the following section.

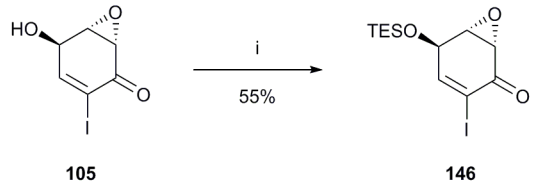


Scheme 2-21: *Proposed synthesis of (−)-epiepoformin from the iodide **105**, a halo-analogue of (−)-bromoxone.*

2.9 TOTAL SYNTHESIS OF (−)-EPIEPOFORMIN

2.9.1 The Stille cross-coupling reaction

In accord with the proposed synthetic plan shown immediately above, alcohol **105** was reacted with triethylsilyl chloride in the presence of 2,6-lutidine (Scheme 2-22), a hindered base used to facilitate the silylation reaction as well as to reduce the incidence of possible competing (base-induced) aromatization processes. The desired reaction was complete after one hour and the substrate for the planned Stille cross-coupling reaction, silyl ether **146**, was thus obtained in 55% yield.



105 **146**

Scheme 2-22: *Reagents and conditions* (i) TES-Cl, 2,6-lutidine, dichloromethane, 0 → 20 °C, 1 h.

The ^{1}H NMR spectrum of compound **146** is shown in Figure 2-13. Signals arising from protons attached to the cyclohexene ring were mostly unchanged with respect to the chemical shifts observed in the analogous spectrum of precursor **105**. The quartet and triplet observed at δ 0.67 and 0.98 arise from the three equivalent ethyl groups associated with the triethylsilyl protecting group.

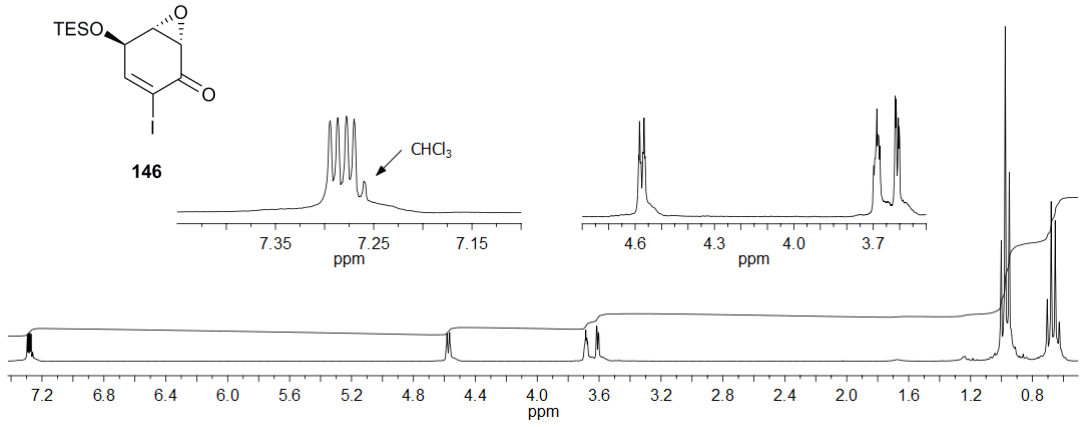
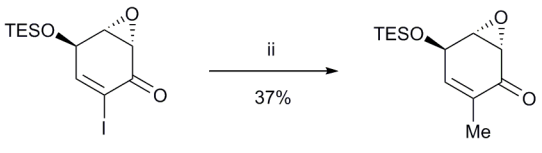


Figure 2-13: *300 MHz ^{1}H NMR spectrum of silyl ether **146** (recorded in $CDCl_3$).*
Inset: Expansions of two regions from the spectrum.

Disappointingly, the foreshadowed palladium-catalyzed cross-coupling reaction of iodide **146** with tetramethyltin proceeded in only 37% yield to afforded ketone **147** (Scheme 2-23). The extended duration of the reaction and the vigorous heating required almost certainly contributed to degradation of the substrate and/or product.



146 **147**

Scheme 2-23: *Reagents and conditions* (i) Me_4Sn, Pd[0], Ph_3As, CuI, THF, 80 °C, 3 days.

The ^{13}C NMR spectrum recorded on silyl ether **147** is shown in Figure 2-14. The newly introduced α-methyl carbon gives rise to a resonance at δ 15.9 while the associated protons give

rise to a three-proton singlet at δ 1.82 in the corresponding ^{1}H NMR spectrum. The EI mass spectrum displayed a molecular ion at *m/z* 254 and an accurate mass measurement on this species established that it was of the expected composition, *viz.* $C_{13}H_{22}O_3Si$.

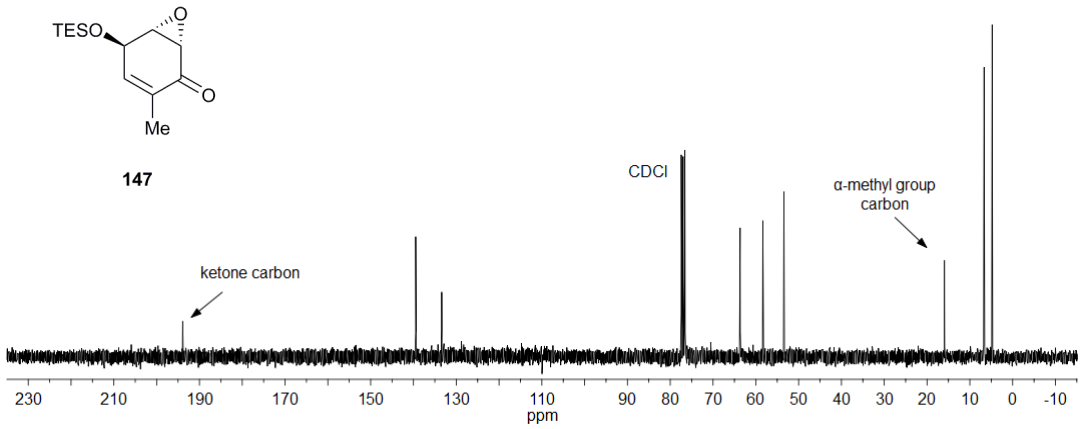
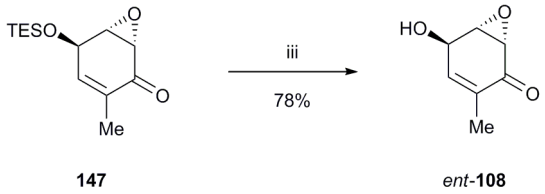


Figure 2-14: *300 MHz ^{13}C NMR spectrum of ketone **147** (recorded in $CDCl_3$).*

As depicted in Scheme 2-24, cleavage of the silyl protecting group of ether **147** with HF/pyridine in acetonitrile furnished the target compound (*ent*-**108**) as a clear, colorless oil. The optical rotation recorded on this material {$[\alpha]_D$ −311.0 (*c* 0.27, ethanol)} was of similar magnitude but opposite sign to that reported[113] for the natural enantiomeric form of epiepoformin {$[\alpha]_D$ +316.04 (*c* 0.37, ethanol)}.



Scheme 2-24: HF·pyridine, MeCN, 20 °C, 1 h.

The ^{13}C NMR spectrum recorded on (−)-epiepoformin is shown in Figure 2-15 and proved to be fully consistent with the assigned structure. Unfortunately, the ^{13}C NMR spectrum of the naturally-derived material has not been published so relevant comparisons could not be made. Nevertheless, our data compare favourably with those reported for synthetically-derived (+)-epiepoformin.

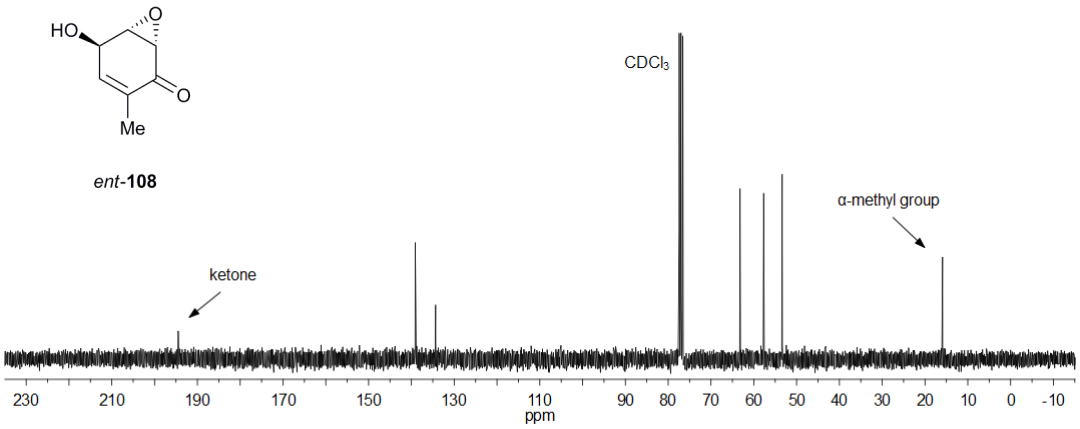


Figure 2-15: *75 MHz ^{13}C NMR spectrum of (−)-epiepoformin.*

A tabulation of the ^{1}H NMR data recorded on both naturally- and synthetically-derived samples of epiepoformin (**108** and *ent*-**108**, respectively) is shown in Table 2-3. Clearly there is close agreement between the two data sets; the maximum deviation between chemical shifts recorded on the synthetic sample, with respect to those reported for the naturally-derived material, was 0.06 ppm and the average deviation was just 0.04 ppm.

Table 2-3: *Comparison of the ^{1}H NMR data recorded on naturally- and synthetically-derived epiepoformin (**108** and* ent-***108**, respectively)*

^{1}H NMR Data Natural **108** (δ_H) (300 MHz, $CDCl_3$)[113]	^{1}H NMR Data Synthetic *ent*-**108** (δ_H) (300 MHz, $CDCl_3$)
6.40 (m, 1H)	6.46 (m, 1H)
4.61 (broad s, 1H)	4.66 (broad m, 1H)
3.73 (m, 1H)	3.79 (m, 1H)
3.46 (d, *J* = 4 Hz, 1H)	3.51 (dd, *J* = 3.6 and 1.2 Hz, 1H)
2.20 (broad s, 1H)	2.20 (broad d, *J* = 7.8 Hz, 1H)
1.82 (s, 3H)	1.84 (broadened s, 3H)

The present work represents the equal shortest synthesis of epiepoformin as is indicated in Table 2-4, and delivers the target compound in an enantiomerically enriched form. The overall yield for this synthesis is low, an outcome that is partly attributed to the vigorous reaction conditions required for installation of the α-methyl moiety. In consideration of this shortcoming, the development of a more efficient cross-coupling regime is considered worthwhile. Conveniently, however, as the starting material for the synthesis, *cis*-dihydrocatechol **100**, is derived from abundant iodobenzene, all but one of the carbons of target **147** are derived this commodity chemical.

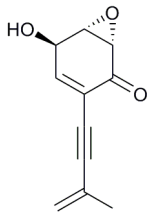
Table 2-4: *A comparison of the key features associated with the seven reported syntheses of epiepoformin*

Lead author	Publication date	Longest linear sequence	Overall yield (%)	Modification produced
Ichihara[84]	1982	seven steps	7	(±)
Ogasawara[85]	1995	twelve steps	37	(+)
Maycock[86]	2000	ten steps	20	(+)
Okamura[87]	2001	nine steps	29	(+)
Kitahara[83]	2003	thirteen steps	10	(+)
Carreno[88]	2005	nine steps	9	(+)
Present work[104]	2009	seven steps	5	(−)

2.10 HARVEYNONE

2.10.1 Isolation and characterisation of harveynone

(−)-Harveynone [**148**, aka (−)-PT toxin] was first isolated from the mold *Curvularia harveyi* and shown, at that time, to inhibit spindle formation in sea urchin eggs.[119] This property is shared by its enantiomer, (+)-harveynone (*ent*-**148**), a phytotoxic metabolite of the tea gray blight fungus *Psetalotiopsis theae*.[120]



148

Figure 2-16: *(−)-Harveynone*

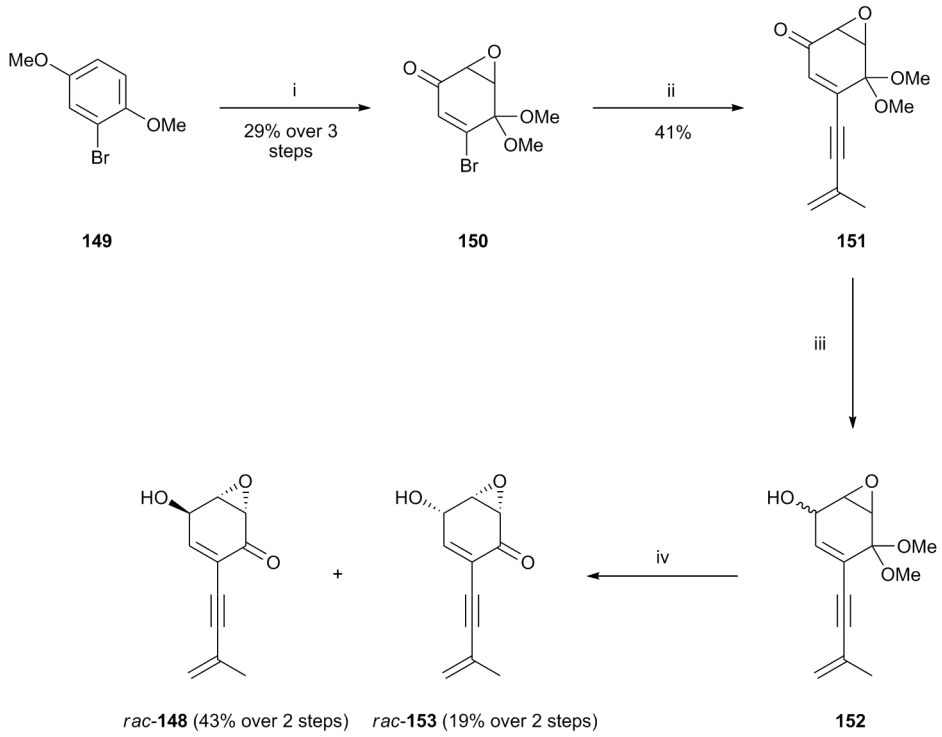
2.10.2 Previous studies on the synthesis of harveynone

The (±), (+) and (−) forms of harveynone have each been the subject of total syntheses, most of which use Pd[0]-catalyzed cross-coupling reactions to install the enynyl side-chain.[86,89-92] However, the most recently reported synthesis of (−)-harveynone used a very different methodology. In particular, a tandem reaction sequence involving relay metathesis-induced enyne ring-closing metathesis and a metallotropic [1,3]-shift reaction were employed. A discussion of each of the reported syntheses is presented immediately below.

Taylor's total syntheses of (±)-harveynone (1996)

By applying Pd[0]-catalyzed cross-coupling reactions to highly functionalized cyclohexenyl halides, Taylor *et al.* generated racemic samples of harveynone and its C-4 epimer *via* two similar routes (Scheme 2-25 and Scheme 2-26).[89] In the first of these, β-bromocyclohexenone

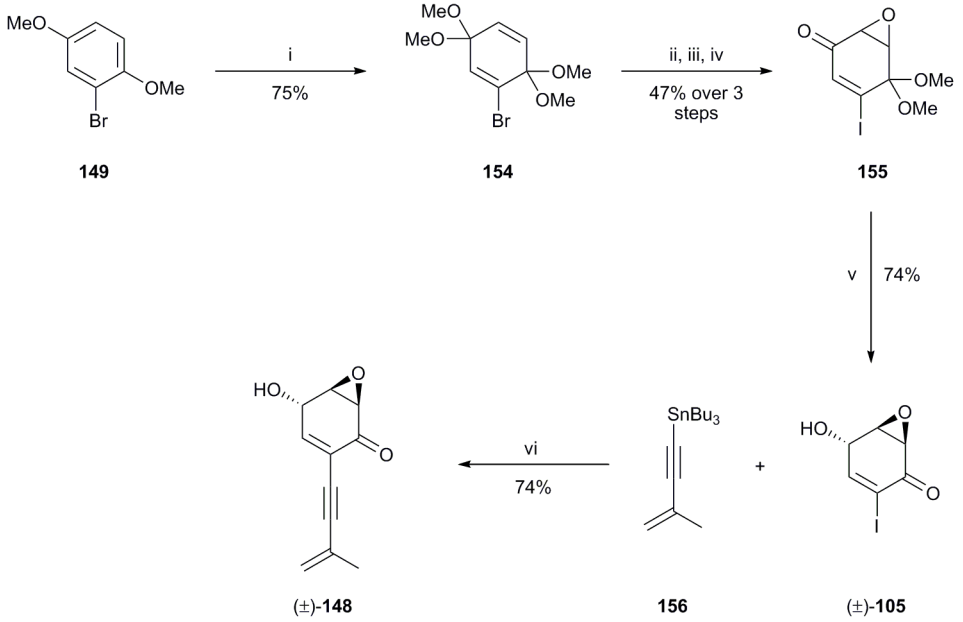
150, which had been used by the same group in a synthesis of bromoxone (Section 2.2.2), was cross-coupled with 2-methyl-1-en-3-yne under Sonogoshira conditions. Di*iso*butylaluminium hydride-mediated reduction of the resulting ketone (**151**) generated a diastereomeric mixture of alcohols **152** and Montmorillonite K10-mediated hydrolysis of the dimethyl ketal moiety in the product delivered the expected mixture of chromatographically separable *trans*- and *cis*-epoxy alcohols (*rac*-**148** and *rac*-**153**, respectively), the former representing (±)-harveynone.



Scheme 2-25: *Reagents* (i) As described by Taylor *et al*.;[79] (ii) 2-methylbut-1-en-3-yne, (*i*-Pr)$_2$NH, Pd(OAc)$_2$, PPh$_3$; (iii) (*i*-Pr)$_2$AlH; (iv) Montrorillonite K10.

A more efficient protocol for the preparation of (±)-harveynone was later devised by the same authors and is shown in Scheme 2-26. In this instance, the Pd[0]-catalyzed cross-coupling reaction of a compound containing the fully elaborated core of (±)-harveynone was used (Scheme 2-26).[121] The same starting material, bromoquinol **149**, was protected as the corresponding dimethyl *bis*-ketal **154** and then treated with *n*-butyllithium and molecular iodine in order to effect a *trans*-halogenation reaction. The less-hindered ketal within the resulting iodide was selectively hydrolyzed with aqueous acetic acid and the enone so-formed was subjected to a facially-selective nucleophilic epoxidation reaction using hydrogen peroxide and sodium hydroxide. In this way, epoxide **155** was obtained. Stereoselective reduction of the ketone moiety within compound **155** could be achieved using either di*iso*butyl aluminium hydride or lithium triethylborohydride. The first of these reagents selectively produced the *trans*-alcohol and, in a complimentary fashion, the latter reagent delivered its epimer. The acetal moiety within the *trans*-reduction product was hydrolyzed in presence of Montmorillonite K10

to give iodide (±)-**105**. Finally, Stille cross-coupling of this substrate with tri-*n*-butyl(3-methylbut-3-en-1-ynyl)stannane (**156**) afforded (±)-harveynone in 43% yield over the final two steps.

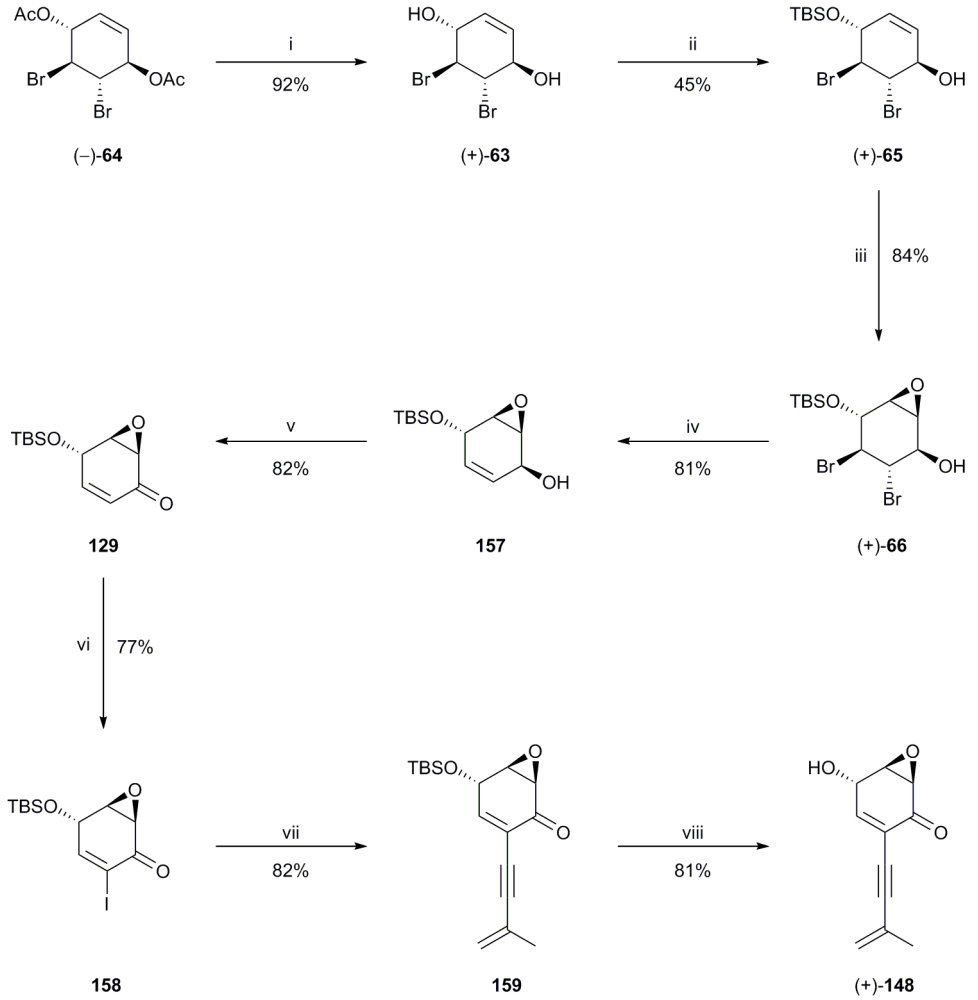


149 **154** **155** (±)-**148** **156** (±)-**105**

Scheme 2-26: *Reagents* (i) anodic oxidation, MeOH; (ii) BuLi, I_2; (iii) aq. AcOH; (iv) H_2O_2, NaOH; (v) $(i\text{-Pr})_2AlH$, Montmorillonite K10; (vi) $PdCl_2(PPh_3)_2$, CuI.

Johnson's total synthesis of (+)-harveynone (1997)

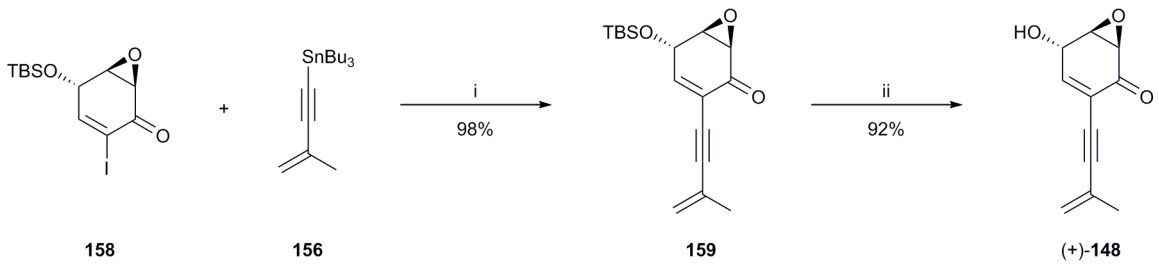
The initial steps of the Johnson synthesis of (+)-harveynone (Scheme 2-27) are common to the route that this group employed in synthesizing (+)-bromoxone (Section 2.2.2).[80,90] Thus, the sequence proceeds from diester (−)-**64**, which was obtained *via* the enzymatic resolution process described earlier.[80] Exposure of this material to titanium(IV) isopropoxide cleaved both esters and afforded diol (+)-**63**. This diol was then mono-protected using *tert*-butyldimethylsilyl triflate and triethylamine to give silyl ether (+)-**65** in 45% yield. Hydroxyl group directed *syn*-epoxidation of ether (+)-**65** gave compound (+)-**66** and reduction of the vicinal bromide within the latter material using zinc metal gave allylic alcohol **157** which was oxidized to enone **129** with PCC. α-Iodination of the last compound with molecular iodine and pyridine gave compound **158** that was then engaged in a Sonogashira cross-coupling reaction with 2-methylbut-1-en-3-yne to give dienyne **159**. Interestingly, this reaction proceeded more rapidly in the presence of di*iso*propylamine than triethylamine. The coupling product **159** so-formed was then deprotected with hexafluorosilicic acid in acetonitrile and thereby giving (+)-harveynone in 12 steps and an overall yield of 1.4% from *p*-benzoquinone (**62**).



Scheme 2-27: *Reagents* (i) Ti(O*i*-Pr)$_4$; (ii) TBSOTf, Et$_3$N; (iii) CF_3CO_3H, Na_2HPO_4; (iv) Zn; (v) PCC; (vi) I_2, pyridine (vii) $PdCl_2(PPh_3)_2$, CuI, *i*-Pr$_2$NH, 2-methylbut-1-en-3-yne; (viii) H_2SiF_6.

Maycock's total synthesis of (+)-harveynone (2000)

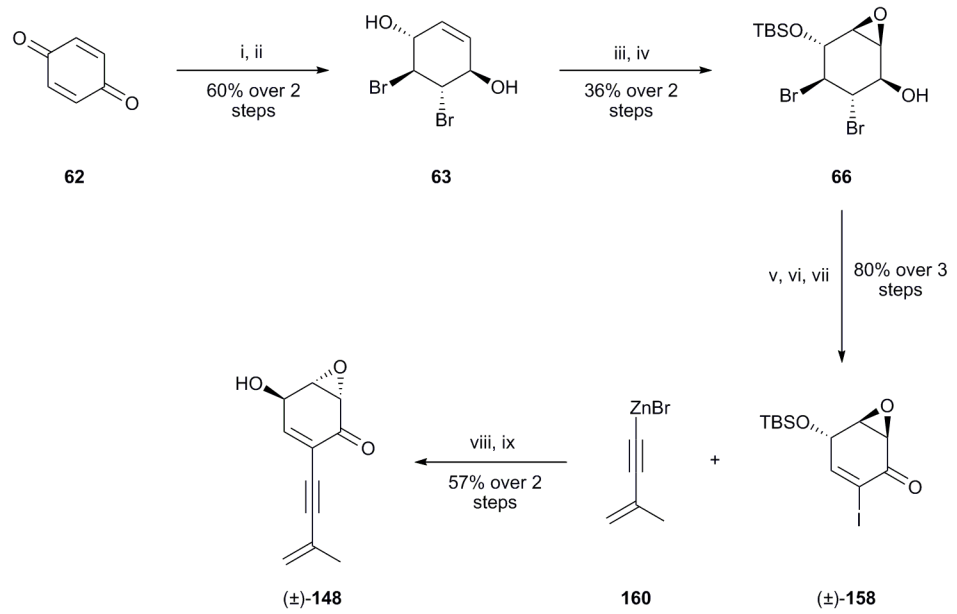
Maycock *et al*. described a synthesis of (+)-harveynone from compound **158**, an intermediate in this synthesis of (+)-epiepoformin and described earlier in this chapter (Section 2.7.2).[86] As depicted in Scheme 2-28, iodide **158** underwent cross-coupling with tri-*n*-butyl(3-methylbut-3-en-1-ynyl)stannane (**156**) under mild conditions to provide target **159** in 98% yield. Deprotection of the cross-coupling product with hydrofluoric acid in acetonitrile then gave (+)-harveynone [(+)-**148**] in 92% yield.



Scheme 2-28: *Reagents* (i) $Pd(PPh_3)_2Cl_2$, $AsPh_3$, CuI; (ii) 40% aq. HF.

Negishi's total synthesis of (±)-harveynone (2000)

Negishi *et al*. applied their signature Pd[0]-catalyzed cross-coupling reaction to a synthesis of (±)-harveynone (Scheme 2-29) and this was published alongside a related synthesis of tricholomenyn A.[91] Thus, *p*-benzoquinone (**62**) was treated with molecular bromine and the resulting dibromo-diketone was reduced to diol **63** with sodium borohydride.[122] Selective monosilylation of this *meso*-compound afforded an intermediate alkene that underwent stereoselective epoxidation upon treatment with the urea/hydrogen peroxide adduct, to afford epoxide **66**.[90,123] A suspension of zinc metal in methanol effected reduction of the vicinal dibromide moiety within compound **66** and the allylic alcohol so-produced was oxidized to the corresponding ketone with PCC. Iodination at the α-position of the ensuing enone was carried out with molecular iodine and pyridine and thereby afforded compound (±)-**158**.[124] The key step in this reaction sequence, the Negishi cross-coupling reaction between alkenyl iodide (±)-**158** and the relevant alkynyl zinc bromide (**160**), proceeded in 73% yield. Desilylation of the resulting coupling product then afforded the target compound [(±)-**148**] in 80% yield.[125]



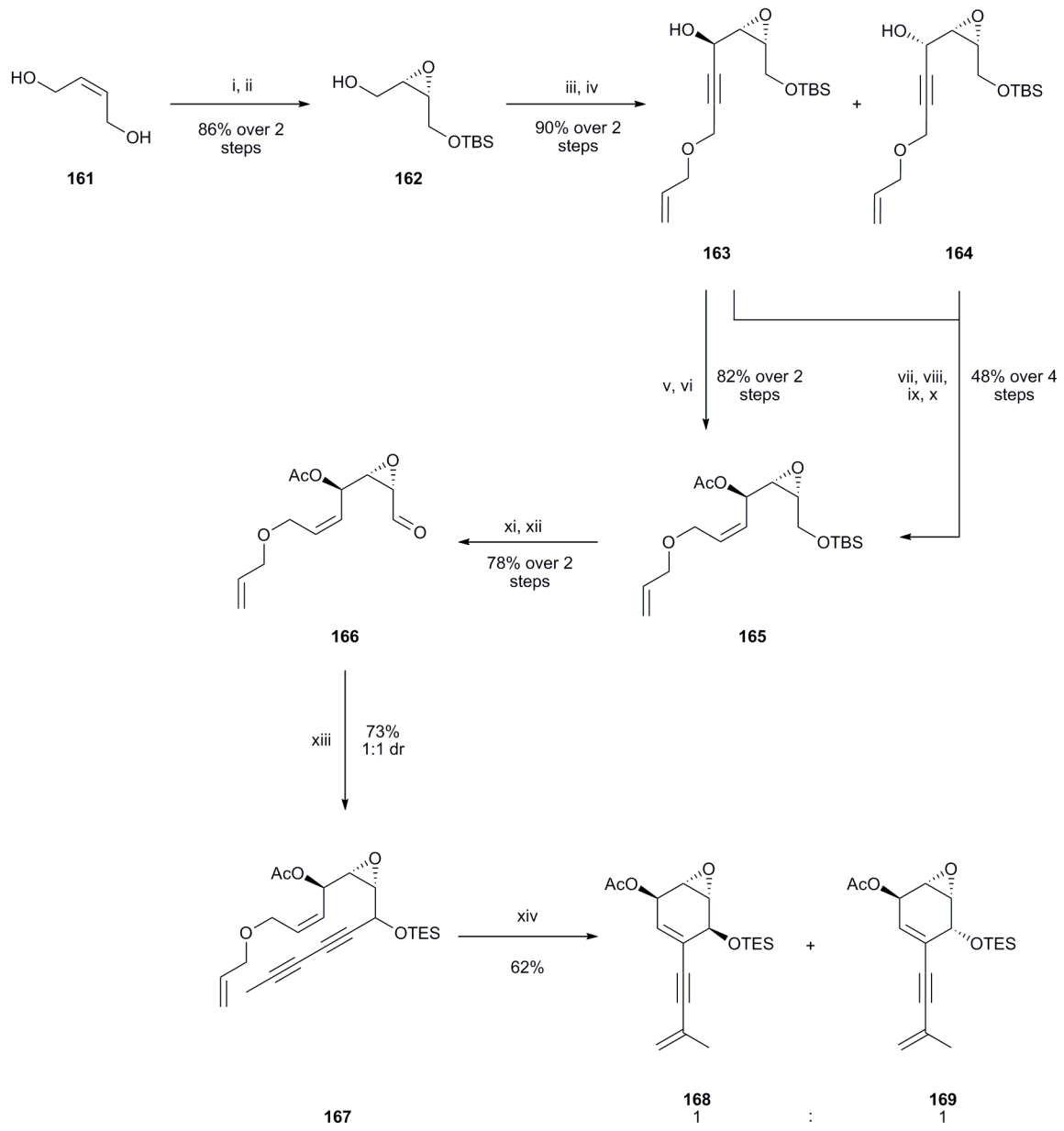
Scheme 2-29: *Reagents* (i) Br_2; (ii) $NaBH_4$; (iii) TBS-Cl, imidazole; (iv) NaH_2PO_4, urea hydrogen peroxide adduct; trifluoroacetic anhydride; (v) zinc; (vi) PCC, Celite; (vii) I_2, pyridine; (viii) $Pd(dba)_2$, tris(2-furyl)phosphine; (ix) 48% hydrofluoric acid (aqueous).

Lee's total synthesis of (−)-harveynone (2009)

Recently, Lee *et al*.[92] published an especially novel approach to (−)-harveynone (Scheme 2-30) and some related natural products. The key steps in these syntheses were a tandem reaction sequence involving relay metathesis-induced enyne ring-closing metathesis and a metallotropic [1,3]-shift. The sequence commenced with mono-*O*-silylation of commercially-available *cis*-2-butene-1,4-diol (**161**) and subsequent Sharpless asymmetric epoxidation of the intermediate ether in a manner described previously.[126-128] Oxidation of the ensuing alcohol (**162**) with PCC

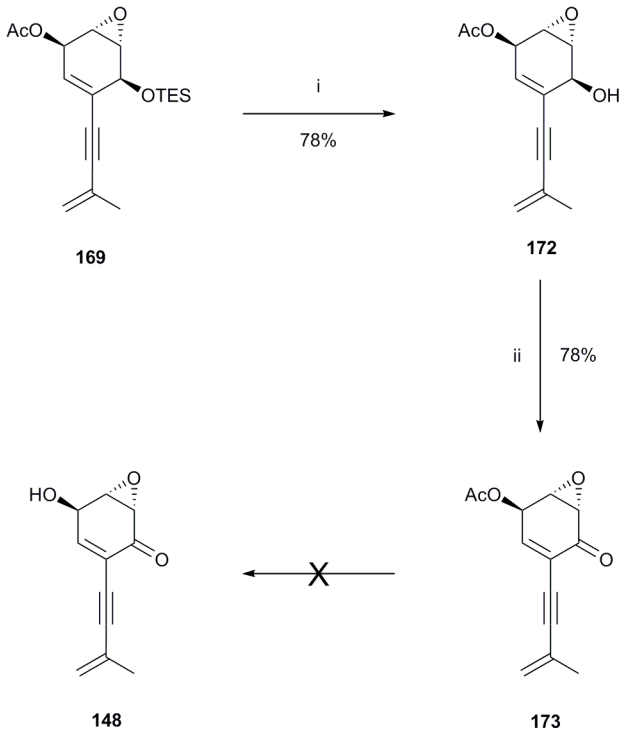
provided an aldehyde that was treated with an acetylide anion and thus generating a 2:1 and chromatographically separable mixture of epimeric alcohols **163** and **164**. β-Epimer **163** was converted into the pivotal acetate **165** *via* sequential Lindlar reduction and acylation steps. A more efficient synthesis of acetate **165** was achieved by oxidation of the epimeric alcohols **163** and **164** with the Dess-Martin periodinane. Corey-Bakshi-Shibata (CBS) reduction of the resulting ketone proceeded diastereoselectively, though the undesired α-epimer predominated. Nevertheless, following Lindlar reduction of these alkynes, and a Mitsunobu reaction, ester **165** was obtained with the desired stereochemistry as shown in Scheme 2-30. Cleavage of the silicon protecting group from compound **165** was effected with HF in pyridine and the corresponding aldehyde **166** was generated from the ensuing alcoholic intermediate *via* oxidation with the Dess-Martin reagent. Exposure of compound **166** to a catalytic quantity of tetra-*n*-butylammonium difluorotriphenylsilicate induced the non-stereoselective attack of a diynyl anion [produced *in situ* from triethyl(penta-1,3-diynyl)silane] on the aforementioned aldehyde. Concomitant transfer of the triethylsilyl group from the diyne to oxygen produced a 1:1 mixture of the epimeric *O*-silyl ethers **167**, the substrates required for the key step of the reaction sequence.

Treatment of a compound **167**, as a dilute solution in dichloromethane, with Grubbs' second-generation catalyst provided the chromatographically separable acetates **168** and **169** containing the 1,5-diene-3-yne substructures common to (−)-harveynone and the related alkaloid (+)-asperpentyn, respectively. This conversion presumably involves initial insertion of the ruthenium species into the vinyl moiety of **167**. The tethered allyl ether serves as a relay device that delivers ruthenium to the *cis*-alkene and thereby forms a new alkylidene. This alkylidene undergoes a ring-closing metathesis reaction with the nearby alkyne to form a third Ru-alkylidene species. From this species, a metallotropic [1,3]-shift delivers a fully conjugated alkylidene. Finally, departure of the ruthenium complex generates the depicted and epimeric mixture of cyclohexenes **168** and **169**, each incorporating the desired alkynyl substituent.



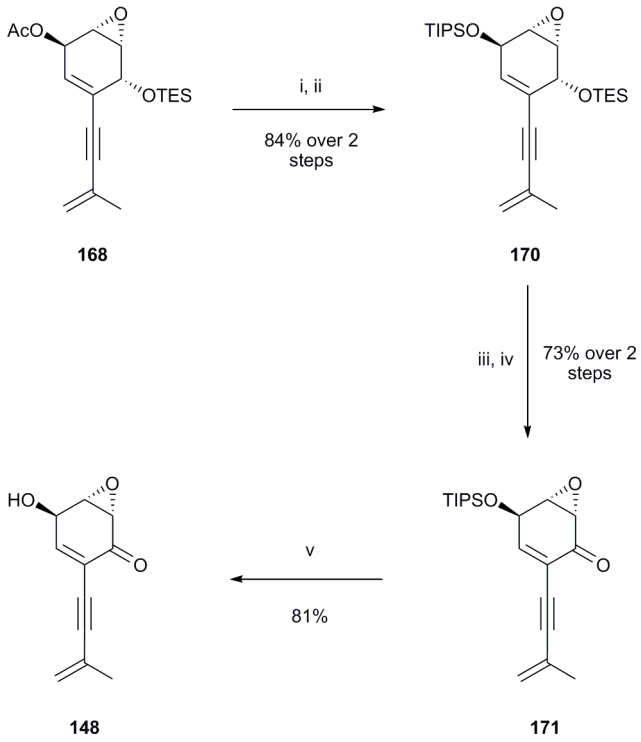
Scheme 2-30: *Reagents* (i) BuLi, TBS-Cl; (ii) (+)-diethyl tartrate, Ti(O*i*-Pr)$_4$, *t*-BuOOH, 4 Å molecular sieves; (iii) PCC, 4 Å molecular sieves; (iv) BuLi, 3-(prop-2-ynyloxy)prop-1-ene; (v) H_2, Pd($CaCO_3$), Pb($OAc)_4$, quinoline; (vi) Ac_2O, pyridine; (vii) DMP; (viii) (*R*)-Me-CBS BH_3SMe_2; (ix) H_2, Pd($CaCO_3$), Pb($OAc)_4$, quinoline; (x) DIAD, PPh_3, AcOH; (xi) HF, pyridine; (xii) DMP; (xiii) triethyl(penta-1,3-diynyl)silane, $Ph_3SiF_2NBu_4$; (xiv) Grubbs II.

(−)-Harveynone was thought to be readily accessible from C1-β-epimer **169**. In an effort to achieve such a conversion (Scheme 2-31), the silyl group of compound **169** was selectively cleaved with hydrofluoric acid in pyridine to reveal allylic alcohol **172** which was then oxidized with manganese (IV) oxide. However, deacetylation of the resulting ketone **173** could not be effected under a range of conditions due to competing aromatization processes, though the structure of such undesired products was not explicitly specified. As such, it appeared necessary to install a protecting group that could be removed under milder conditions prior to establishing the epoxyquinol scaffold.



Scheme 2-31: *Reagents* (i) HF·pyridine; (ii) MnO_2.

Conveniently for Lee *et al.*, the C1-α-epimer **168** underwent selective cleavage of its appended acetate ester upon its exposure to potassium cyanide and the liberated alcohol was then immediately reprotected as the corresponding tri*iso*propylsilyl ether, *viz.* compound **170** (Scheme 2-32). Hydrofluoric acid in pyridine was then used to effect deprotection of the less sterically encumbered triethylsilyl protecting group. A MnO_2-mediated oxidation of the resulting alcohol then afforded ketone **171**. A second desilylation reaction, now using hydrofluoric acid in acetonitrile, completed this particularly novel route to (−)-harveynone which proceeded in 3% overall yield and 17 steps from diol **161**.

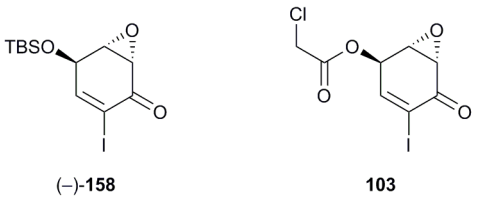


Scheme 2-32: *Reagents* (i) KCN; (ii) TIPSOTf, Et_3N; (iii) HF·pyridine; (iv) MnO_2; (v) HF·pyridine.

2.11 SYNTHETIC STRATEGY ASSOCIATED WITH THE PRESENT SYNTHESIS OF (−)-HARVEYNONE

2.11.1 Proposed synthetic route to (−)-harveynone

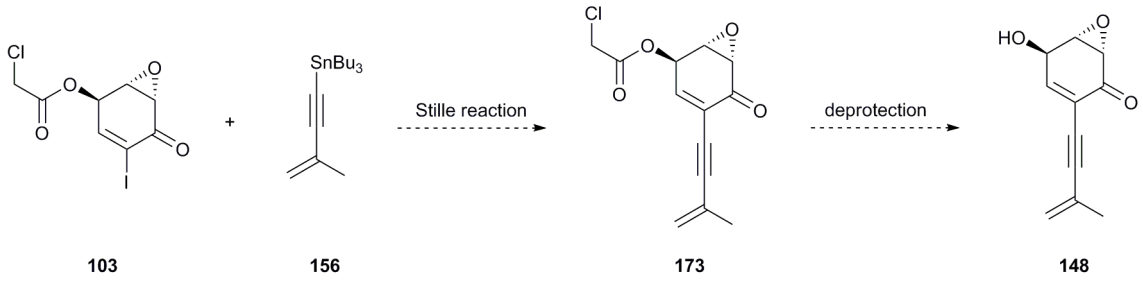
For all of the aforementioned total syntheses of harveynone,[86,89-91] except that reported by Lee *et al.*,[92] Pd[0]-catalyzed cross-coupling reactions have been used to introduce the enynyl side-chain of the natural product, usually in either the last or second-to-last step of the relevant reaction sequence. The most frequently employed halide-coupling partner for these late-stage cross-coupling reactions was silyl ether **158**. Advantageously, the preparation of ester **103** had already been carried out earlier *en route* to the iodo-congener (**105**) of (−)-bromoxone. Thus, as described in Section 2.3, ester **103** is readily prepared from the plentiful and enantiomerically pure *cis*-dihydrocatechol **90** in just four steps. This ester (**103**) bears an α-chloroacetyl protecting group instead of the more usual TBS group as encountered in the proven precursor, **158**, to (−)-harveynone.



(–)-**158** **103**

Figure 2-17: α-Iodo cyclohexenones (−)-**158** and **103**.

It was proposed that iodide **103** would serve as a suitable coupling partner with which to engage stannane **156** in a Stille cross-coupling reaction (Scheme 2-33). It was also expected that the α-chloroacetate ester would be readily cleaved from the target compound in a fashion analogous to that applied earlier for the synthesis of (−)-bromoxone and its iodo-congener. The successful implementation of such a sequence is described in the following section.



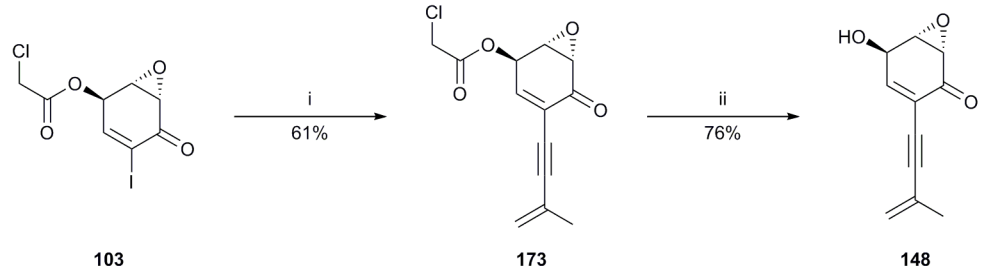
103 **156** **173** **148**

Scheme 2-33: *The proposed synthesis of (−)-harveynone.*

2.12 TOTAL SYNTHESIS OF (−)-HARVEYNONE

2.12.1 The Stille cross-coupling reaction

In accord with the proposed route described immediately above, iodide **103**, appended with the α-chloroacetyl protecting group was deployed in the Pd[0]-catalyzed cross-coupling reaction depicted in Scheme 2-34. The requisite stannane (**156**) was prepared from 2-methylbut-1-en-3-yne according to the procedure of Lopez *et al.*[89,129] The Stille cross-coupling reaction of compounds **103** and **156** afforded dienynyl ketone **173** in 61% yield and was complete in just one hour at 20 °C. Treatment of product **173** with zinc (II) acetate in methanol cleaved the α-chloroacetyl group and delivered (−)-harveynone (**148**) in 76% yield. The smooth cleavage of the α-chloroacetyl protecting group highlights its usefulness as a more labile alternative to the typical acetyl ester. The α-chloroacetyl protecting group, selectively installed using the Mitsunobu reaction and later removed under mild conditions, has proven particularly useful for the synthesis of a range of epoxyquinol natural products. This feature was articulated in a 2009 publication that documented this preparation of (−)-harveynone and the preparation of some other epoxyquinol and dimeric epoxyquinol-derived natural products.[103]



Scheme 2-34: *Reagents and conditions* (i) Pd[0], Ph_3As, CuI, stannane **156**, THF, 20 °C, 1 h; (ii) $Zn(OAc)_2.2H_2O$, MeOH, 20 °C, 1 h.

The spectral data recorded on (−)-harveynone (**148**) displayed all of the expected features and are in full accord with the data reported for the natural product. The hydroxyl group of compound **148** gives rise to an absorption band at 3235 cm^{-1} in the IR spectrum while mass spectrometric analysis of this material revealed a molecular ion at *m/z* 190 and an accurate mass measurement on this species indicated that it possessed the expected molecular formula, *viz.* $C_{11}H_{10}O_3$. Furthermore, the specific rotation of this material {$[\alpha]_D$ −204.9 (*c* 0.89, methanol)} was of similar magnitude but of opposite sign to that reported[86] for its optical antipode, (+)-harveynone {$[\alpha]_D$ +206.6 (*c* 0.38, methanol)}.

The ^{13}C NMR spectrum recorded on synthetic (−)-harveynone (**148**) (Figure 2-18) was in complete agreement with that reported for the natural product. The resonance due to the ketonic carbon (C1) is clearly visible at δ 191.5, while resonances arising from the acetylenic carbons C7 and C8 appear at δ 81.2 and 95.9, respectively.

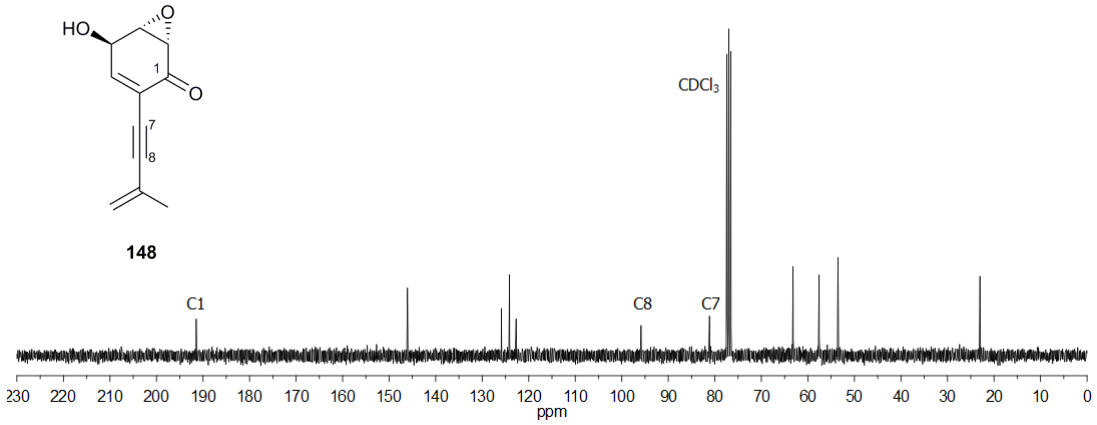


Figure 2-18: *75 MHz ^{13}C NMR spectrum of (−)-harveynone (recorded in $CDCl_3$).*

A comparison of the ^{13}C and ^{1}H NMR data obtained on naturally- and synthetically-derived samples of harveynone (*ent*-**148** and **148**, respectively) is shown in Table 2-5. Differences between the two ^{13}C NMR spectra are no greater than 0.6 ppm in any instance and the average difference is just 0.16 ppm. The ^{1}H NMR spectra are in good agreement, except for the

chemical shift of the hydroxyl proton. The signal arising from this proton appears at δ 2.19 in the spectrum of the naturally-derived material and is shifted to δ 3.00 in the spectrum derived from the synthetically-derived material. This may be due to subtle differences in acidity or concentrations of the samples. The remaining signals, however, are in close accord; the average difference between the relevant signals is just 0.02 ppm.

Table 2-5: *Comparison of the ^{13}C and ^{1}H NMR data recorded on naturally- and synthetically-derived harveynone (*ent-***148** *and* **148***, respectively)*

^{13}C NMR Data Natural *ent*-**148** (δ_C) (68 MHz, $CDCl_3$)[120]	^{13}C NMR Data Synthetic **148** (δ_C) (75 MHz, $CDCl_3$)[103]	^{1}H NMR Data Natural *ent*-**148** (δ_H) (270 MHz, $CDCl_3$)[120]	^{1}H NMR Data Synthetic **148** (δ_H) (300 MHz, $CDCl_3$)[103]
191.0	191.5	6.86 (dd, *J* = 4.9 and 2.4 Hz, 1H)	6.85 (dd, *J* = 5.4 and 2.7 Hz, 1H)
145.6	146.1	5.44 (m, 1H)	5.43 (m, 1H)
125.9	125.9	5.35 (m, 1H)	5.35 (m, 1H)
124.1	124.1	4.78 (m, 1H)	4.73 (broad m, 1H)
122.1	122.7	3.82 (m, 1H)	3.85 (m, 1H)
95.9	95.9	3.58 (dd, *J* = 3.4 and 1.0 Hz, 1H)	3.55 (dd, *J* = 3.6 and 1.2 Hz, 1H)
81.2	81.2	2.19 (d, *J* = 8.8 Hz, 1H)	3.00 (broad d, *J* = 9.3 Hz, 1H)
63.3	63.2	1.94 (s, 3H)	1.93 (s, 3H)
57.5	57.6		
53.5	53.5		
23.0	23.0		

As is revealed in Table 2-6, the present work represents the shortest total synthesis of enantio-enriched harveynone. The overall yield for this synthesis is comparable to that achieved by Maycock *et al*.[86] As the starting material for the synthesis described here, *cis*-dihydrocatechol **100**, is derived from abundant iodobenzene, all but five of the carbon atoms that comprise target **148** are derived from this commodity chemical.

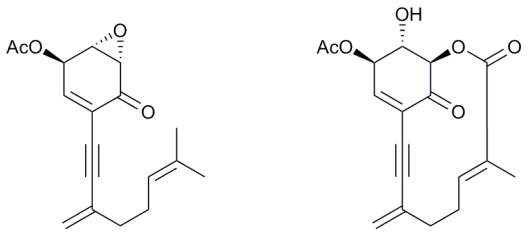
Table 2-6: *A comparison of the key features associated with the six reported syntheses of harveynone*

Lead author	Publication date	Longest linear sequence	Overall yield (%)	Modification produced
Taylor[89]	1996	six steps	5	(±)
Johnson[90]	1997	twelve steps	1	(+)
Maycock[86]	2000	ten steps	20	(+)
Negishi[91]	2000	eight steps	10	(±)
Lee[92]	2009	seventeen steps	3	(−)
Present work[104]	2009	six steps	18	(−)

2.13 (−)-TRICHOLOMENYN A

2.13.1 Isolation and characterization of (−)-tricholomenyn A

In 1995, Garlaschelli *et al*. described the isolation of two new compounds from the fruiting bodies of *Tricholoma acerbum*, a fungus collected from the Italian Appennines.[130] The basic structures of these compounds (see Figure 2-19), designated tricholomenyn A and B (**174** and **175**, respectively), were established using NMR spectroscopic and mass spectrometric techniques. Circular dichroism was used to assign the absolute stereochemistry of tricholomenyn A, the first natural product in the class to incorporate the geranyl acetylenic side-chain.



174 **175**

Figure 2-19: *(−)-Tricholomenyn A* ***(174)*** *and (−)-Tricholomenyn B* ***(175)****.*

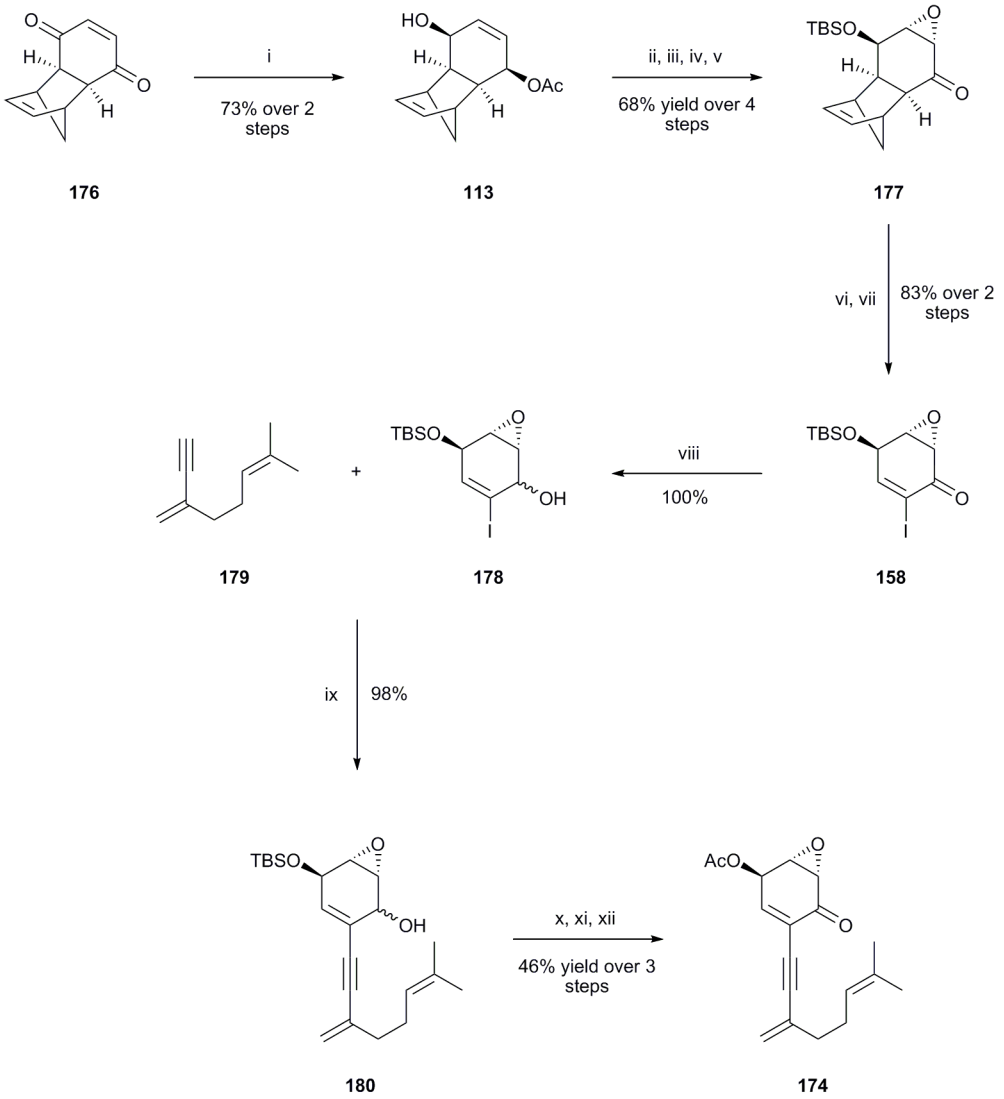
Various tests indicated that tricholomenyn A and B are potent anti-mitotic agents. Thus, when tricholomenyn A was assayed with T lymphocyte cultures, after two hours just 3.1% of cultures underwent mitosis compared with 5-6% of Colcemid™-treated cultures.[130]

2.13.2 Previous studies on the synthesis of tricholomenyn A

Due to interest in the biological properties of tricholomenyn A as well as its densely functionalized cyclohexene framework, this compound has been the subject of a number of total syntheses. Due to its structural similarity to harveynone, the strategies used to synthesize tricholomenyn A are reminiscent of those described in Section 2.10.2. Accordingly, palladium[0]-catalyzed cross-coupling of a cyclohexenyl halide with an acetylenic partner has been used to construct the requisite dienynyl framework. The research groups of Ogasawara, Taylor, Johnson and Negishi each used similar palladium[0]-catalyzed cross-coupling reactions to effect the key C6-C1′ bond-forming event.[90,91,101,102] A recent total synthesis of (−)-tricholomenyn A by Lee *et al*. diverges from these earlier approaches in that relay metathesis-induced enyne ring closing metathesis and metallotropic [1,3]-shift reactions are used to simultaneously establish the cyclohexene framework and the pendant enynyl residue.[92] Each of the reported syntheses of tricholomenyn A is described in detail in the following sections.

Ogasawara's total synthesis of (−)-tricholomenyn A (1996)

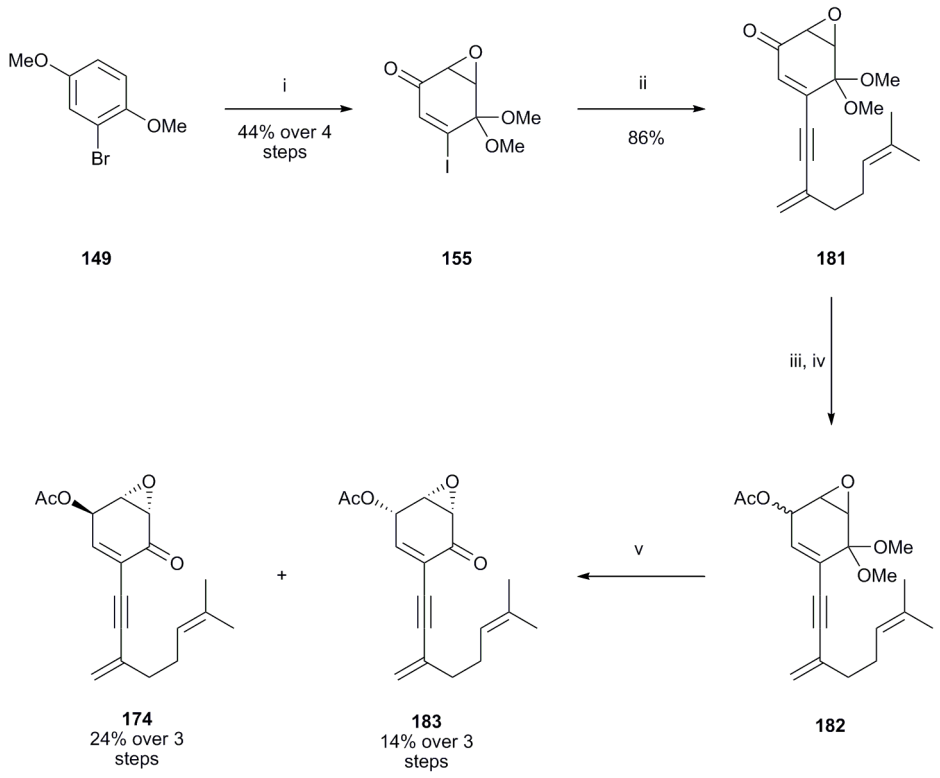
Ogasawara *et al*. employed the chiral acetate **113** in their synthesis of (−)-tricholomenyn A (Scheme 2-35). This acetate was obtained from the reduction of commercially-available Diels-Alder adduct **176** followed by an enzymatic desymmetrization reaction. The free allylic alcohol unit within compound **113** so-obtained was protected as the corresponding *tert*-butyldimethylsilyl ether and the acetate moiety was then cleaved with potassium carbonate to reveal an allylic alcohol. Chromium (VI)-mediated oxidation of this alcohol afforded an intermediate α,β-unsaturated ketone that was subjected to nucleophilic epoxidation using aqueous hydrogen peroxide and Triton B. Heating the ensuing tricycle (**177**) in diphenyl ether induced a retro-Diels-Alder reaction that revealed the previously masked olefin. The ensuing enone was α-iodinated with molecular iodine and pyridine to afford compound **158**. In order to circumvent known difficulties associated with the conduct of Sonogoshira reactions with α-halogenated substrates,[101,131] these researchers opted to reduce the ketone moiety with sodium borohydride with the intention of restoring it at a later stage of the synthesis. This reduction proceeded smoothly and the subsequent Sonogoshira cross-coupling reaction between iodide **178** and alkyne **179** proceeded in a nearly quantitative yield to afford dienyne **180**. As foreshadowed, the ketone moiety was restored by exposing alcohol **180** to the Dess-Martin periodinane. Removal of the silicon-based protecting group from the ensuing enone and subsequent *O*-acetylation under standard conditions revealed the natural product (**174**) in 11 steps and in an overall yield of 18% from adduct **176**.



Scheme 2-35: *Reagents* (i) As described by Takano *et al.*;[115] (ii) TBS-Cl, imidazole; (iii) K_2CO_3, MeOH; (iv) PDC; (v) 30 % aq. H_2O_2, Triton B; (vi) diphenyl ether, reflux; (vii) iodine, pyridine; (viii) $NaBH_4$, $CeCl_3.7H_2O$; (ix) $PdCl_2(PPh_3)_2$, CuI, Et_3N; (x) DMP; (xi) 46 % aq. HF; (xii) Ac_2O, pyridine.

Taylor's total synthesis of (±)-tricholomenyn A (1997)

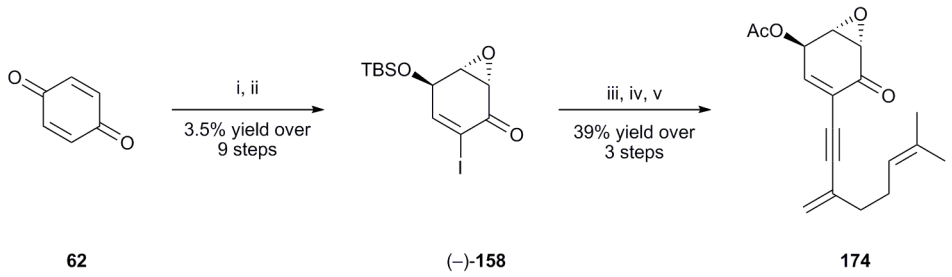
Taylor *et al.* subjected dimethyl ketal **155**, the synthesis of which has been described previously (see Section 2.10.2),[89] to a Pd[0]-catalyzed cross-coupling reaction with trimethyl(7-methyl-3-methyleneoct-6-en-1-ynyl)silane (Scheme 2-36).[89,102] The highly-conjugated dienynone product **181** was reduced and the ensuing mixture of epimeric alcohols was acylated to afford compound **182**. Hydrolysis of the associated ketal unit, using Montmorillonite K10 clay, established the requisite epoxy ketone moiety and afforded a mixture of epimeric esters, the major one being the chromatographically separable tricholomenyn A (*ent*-**174**). In this way, the natural product was obtained in eight steps and in an overall yield of 7% from compound **149**.



Scheme 2-36: *Reagents* (i) As described by Taylor *et al*.;[89] (ii) trimethyl(7-methyl-3-methyleneoct-6-en-1-ynyl)silane, TBAF-SiO_2, $Pd(OAc)_2$, CuI; (iii) DIBAL-H; (iv) Ac_2O, pyridine; (v) Montmorillonite K10.

Johnson's total synthesis of (−)-tricholomenyn A (1997)

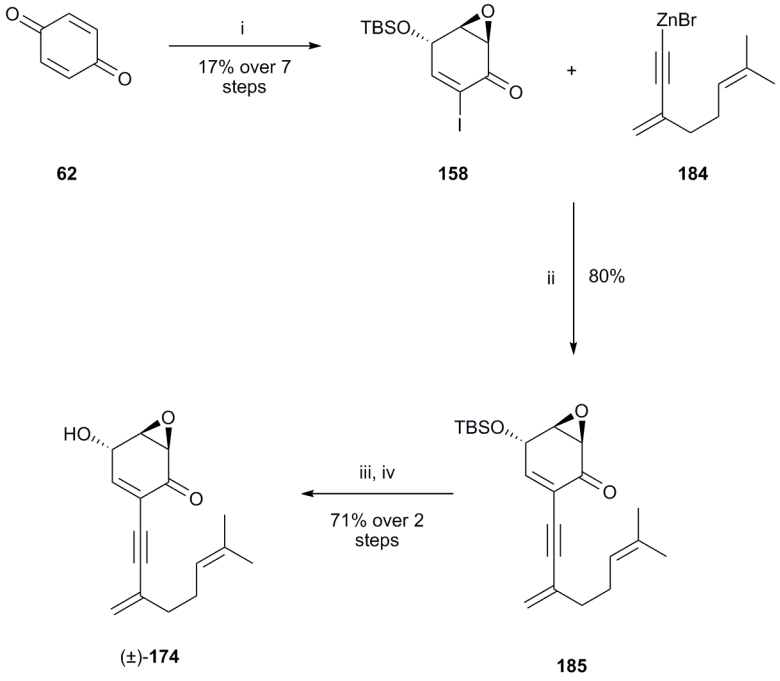
In 1997, Johnson *et al.* described the synthesis of (−)-tricholomenyn A alongside closely related routes to (+)- and (−)-harveynone.[90] By applying an enzymatic resolution step, these researchers were able to obtain the compound (−)-**158** in enantiomerically pure form and 3.5% yield over nine steps.[80,90] A detailed discussion of the preliminary steps of this sequence have been provided in Sections 2.2.2 and 2.10.2. α-Iodo-enone (−)-**158** was engaged in a Sonogoshira cross-coupling reaction with 7-methyl-3-methyleneoct-6-en-1-yne (Scheme 2-37) and the product of this reaction, which was obtained in 54% yield, was desilylated with hexafluorosilicic acid. Steglich esterification of the resulting alcohol using acetic acid introduced the requisite acetyl moiety and thus providing (−)-tricholomenyn A (**174**) in 1.4% yield over 12 steps from *p*-benzoquinone (**62**).



Scheme 2-37 *Reagents* (i) As described by Johnson and Miller;[80] (ii) As described by Johnson and Miller;[90] (iii) $PdCl_2(PPh_3)_2$, CuI, *i*-Pr_2NH, **179**; (iv) H_2SiF_6; (v) DCC, AcOH.

Negishi's total synthesis of (±)-tricholomenyn A (2000)

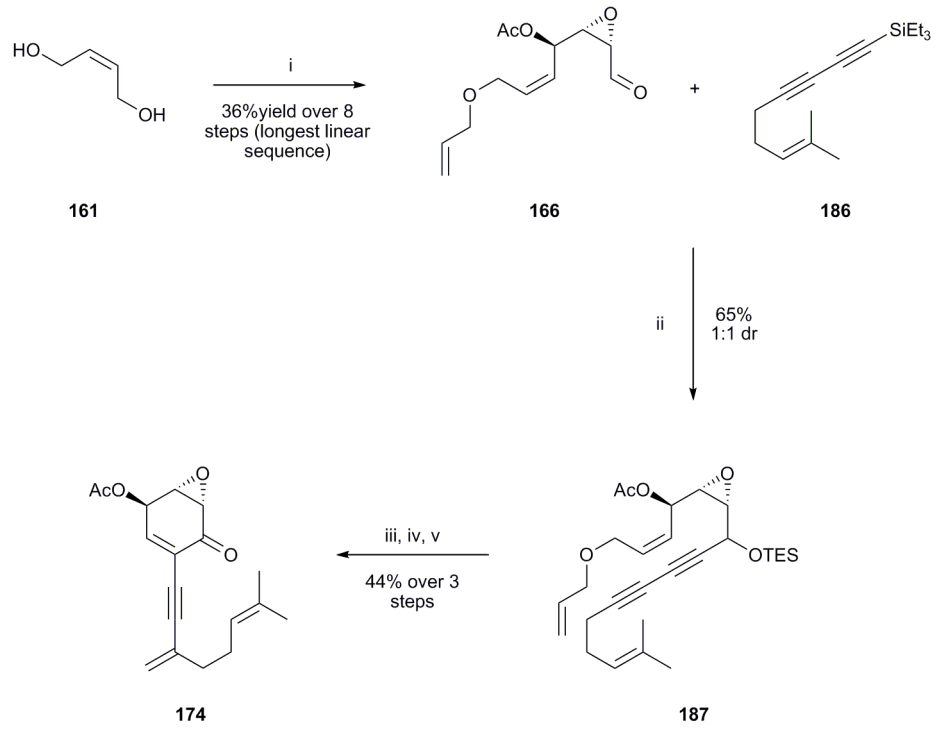
The synthesis of (±)-tricholemenyn A reported by Negishi *et al*. (Scheme 2-38) is closely related to the same group's preparation of (±)-harveynone.[91] Thus, alkenyl iodide **158** is a common intermediate and was obtained as the racemate in seven steps from quinone. Compound **158** was cross-coupled with the relevant alkynyl zinc bromide (**184**) in the presence of a Pd[0] catalyst and tris(2-furyl)phosphine. The silyl ether residue associated with product **185** was cleaved with hydrofluoric acid and the ensuing alcohol was then acetylated (with *N*,*N*'-dicyclohexylcarbodiimide and acetic acid in the presence of dimethylaminopyridine as catalyst) to give (±)-tricholomenyn A in overall yield of 10% and in ten steps from *p*-benzoquinone (**62**).



Scheme 2-38: *Reagents* (i) As described by Negishi *et al*.;[91] (ii) $Pd(dba)_2$, tris(2-furyl)phosphine; (iii) 48% hydrofluoric acid (aqueous); (iv) DCC, DMAP, AcOH.

Lee's total synthesis of (−)-tricholomenyn A (2009)

Lee *et al*. were successful in generating (−)-tricholomenyn A (Scheme 2-39) from aldehyde **166**, an intermediate associated with their related synthesis of (−)-harveynone. Once again, it was a relay metathesis-induced enyne ring-closing metathesis and a subsequent metallotropic [1,3]-shift reaction that featured as the pivotal transformations in the preparation of the target natural product. In order to prepare the substrate for this key reaction, the previously described aldehyde **166** was reacted with diyne **186** in the presence of a catalytic quantity of DeShong's silicate. Upon exposure to the Grubbs' second-generation catalyst the ensuing silyl ether, **189**, underwent the anticipated metathesis and [1,3]-shift reactions to afford the requisite carbon framework of the target natural product. Subsequent desilylation of the enynyl intermediate and oxidation of the alcohol so-formed with MnO_2 yielded (−)-tricholomenyn A (**174**) in 12 steps and an overall yield of 10% from diol **161**.



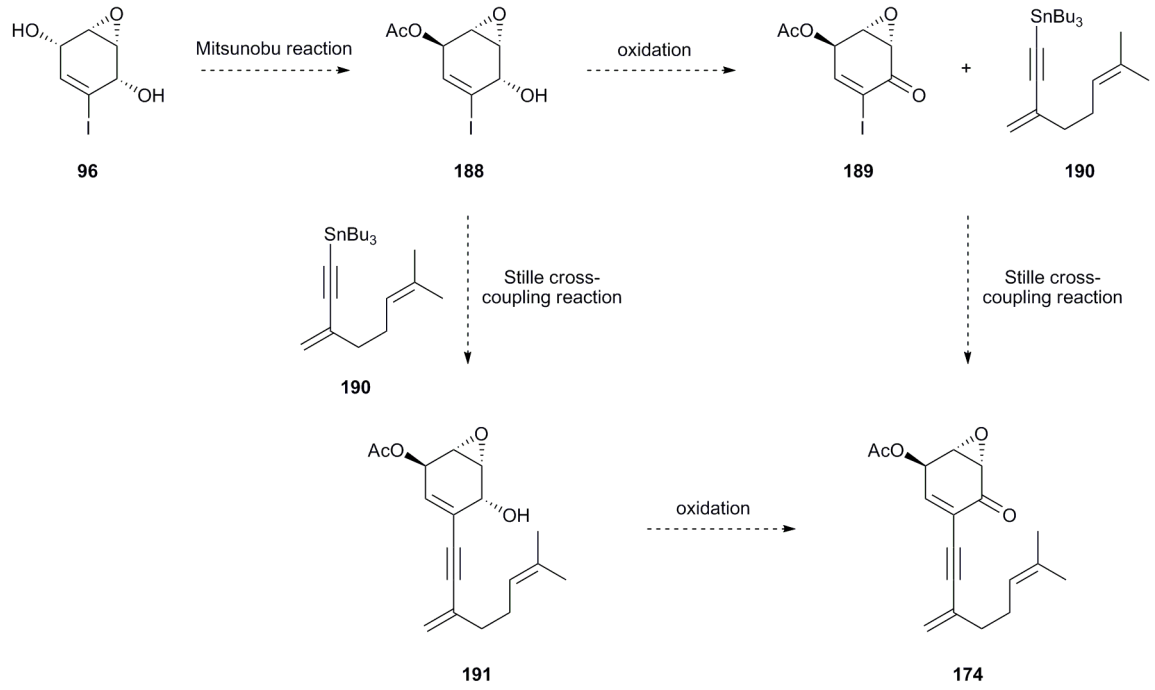
Scheme 2-39: *Reagents* (i) As described by Lee *et al*.;[92] (ii) $Ph_3SiF_2NBu_4$; (iii) Grubbs II; (iv) HF·pyridine; (v) MnO_2.

2.14 SYNTHETIC STRATEGY ASSOCIATED WITH THE PRESENT SYNTHESIS OF (−)-TRICHOLOMENYN A

2.14.1 Proposed route to (−)-tricholomenyn A

It was anticipated that implementation of a cross-coupling strategy analogous to that described earlier for the synthesis of (−)-harveynone would provide a concise and very effective route to

(−)-tricholomenyn A. The relevant strategy is outlined in Scheme 2-40. Thus, Mitsunobu reaction of iodo-diol **96**, itself available in just two steps from *cis*-dihydrocatechol **90**, with acetic acid was expected to establish the necessary acetate moiety with the desired stereochemistry. Oxidation of the allylic alcohol **188** so-formed would then give the corresponding ketone **189**. Cross-coupling of this with the relevant alkynyl stannane **190** should then furnish the target compound. Given the low reactivity of α-haloenones in cross-coupling reactions, reversing these final two steps might provide a more efficient route to (−)-tricholomenyn A.[101,131] As revealed in the following sections, this proposed route proved effective.



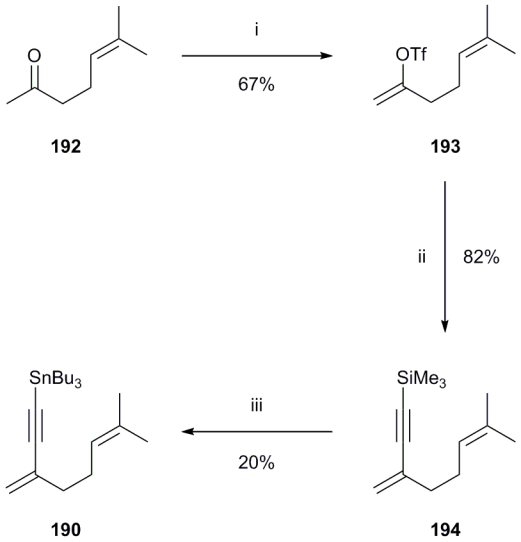
Scheme 2-40: *Proposed synthetic route to (−)-tricholomenyn A.*

2.15 TOTAL SYNTHESIS OF (−)-TRICHOLOMENYN A

2.15.1 Preparation of the relevant alkynyl stannane

The alkynyl stannane **190**, required for the foreshadowed Stille cross-coupling reaction, was obtained in four steps from commercially available prenyl acetone (**192**) (Scheme 2-41). Thus, the kinetic enolate generated from this ketone was trapped with phenyl triflimide to give the previously reported enol triflate **193**.[90,101,102] Sonogashira cross-coupling of compound **193** with commercially available trimethylsilyl acetylene afforded the anticipated dienyne **194** as a pale-yellow oil in 82% yield.[101,102] Under conditions defined by Buchwald,[132] specifically the application of *bis*(tri-*n*-butyltin)oxide and a catalytic amount of tetra-*n*-butylammonium fluoride, silane **194** underwent transmetallation from silicon to tin and thereby giving the

required alkynyl stannane.[101,102] Although the yield for this final step was only 20%, sufficient quantities of stannane **190** could be accumulated by such means to allow an investigation of the pivotal cross-coupling process.



Scheme 2-41: *Reagents and conditions* (i) LDA, THF/hexane, -78 °C, 1 h; $PhN(OTf)_2$, −78 °C to 20 °C, 20 h; (ii) $PdCl_2(NCMe)_2$, CuI, trimethylsilyl acetylene, morpholine/THF, 20 °C, 2 h; (iii) $(n\text{-}Bu_3Sn)_2O$, Bu_4NF, THF, 66 °C, 1 h.

The ^{13}C NMR spectrum recorded on stannane **190** (Figure 2-20) exhibits six low-field signals that correspond to the four olefinic and two acetylenic carbon atoms present in the compound. The IR spectrum of this compound exhibits a sharp absorption band at 2124 cm^{-1} that is attributed to C≡C bond stretching and is intensified by the conjugation of this moiety with the nearby olefin. In the same spectrum, prominent absorption bands arising from sp^3 C−H stretching and deformation are observed at 2958 and 1455 cm^{-1}, respectively.

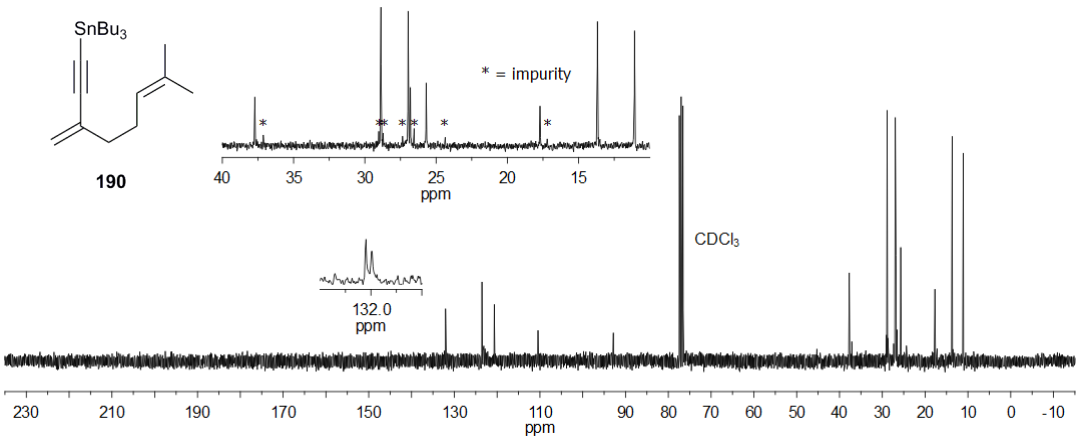
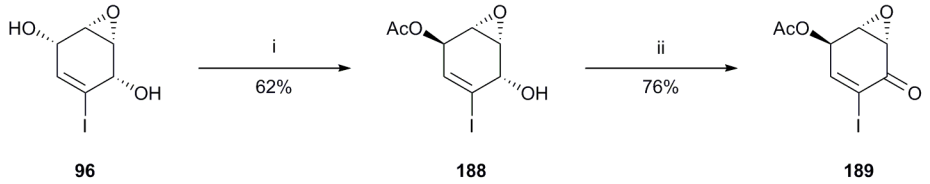


Figure 2-20: 75 MHz *^{13}C NMR spectrum of alkynyl stannane **190** (recorded in $CDCl_3$).*
Insets: Expansions of two regions of the spectrum.

2.15.2 Preparation of acetate cross-coupling partners

In accord with the planned synthesis of (−)-tricholomenyn A (Scheme 2-42), iodo-diol **96** was submitted to a Mitsunobu reaction with acetic acid. As described in Section 2.3.4, these substrates undergo this type of reaction regioselectively. Specifically, the less sterically encumbered hydroxyl group reacts preferentially under these conditions to form a single mono-acetate in a yield of 62%. No traces of alternate isomers or diacetate by-products were detected following concentration of the reaction mixture. The allylic alcohol **188** obtained from the Mitsunobu reaction was oxidized with pyridinium dichromate and the α-iodo enone **189** was thereby obtained in 76% yield.



96 **188** **189**

Scheme 2-42: *Reagents and conditions* (i) (*t*-BuOCON)$_2$, PPh$_3$, AcOH, THF, 20 °C, 0.25 h; (ii) PDC, AcOH, DCM, 20 °C, 1h.

The ^{1}H NMR (see Figure 2-21) and ^{13}C NMR spectra recorded on alcohol **188** each exhibited a close resemblance to the analogous spectra described earlier for the α-chloroacetyl analogue **100**. Thus, the olefinic proton gives rise to a resonance at δ 6.40 which exhibited a multiplicity indicative of its interaction with the three other protons attached to the cyclohexene ring. Protons of the appended acetate residue resonated as a three-proton singlet at δ 2.08. The ester moiety was evident through the appearance of strong absorption bands at 1727 and 1236 cm^{-1}. The hydroxyl residue also produced a strong absorption band at 3468 cm^{-1}. The EI mass spectrum of alcohol **188** displayed a molecular ion at *m/z* 296 and an accurate mass measurement of this species established that it possessed the expected molecular formula, *viz*. $C_8H_9IO_4$.

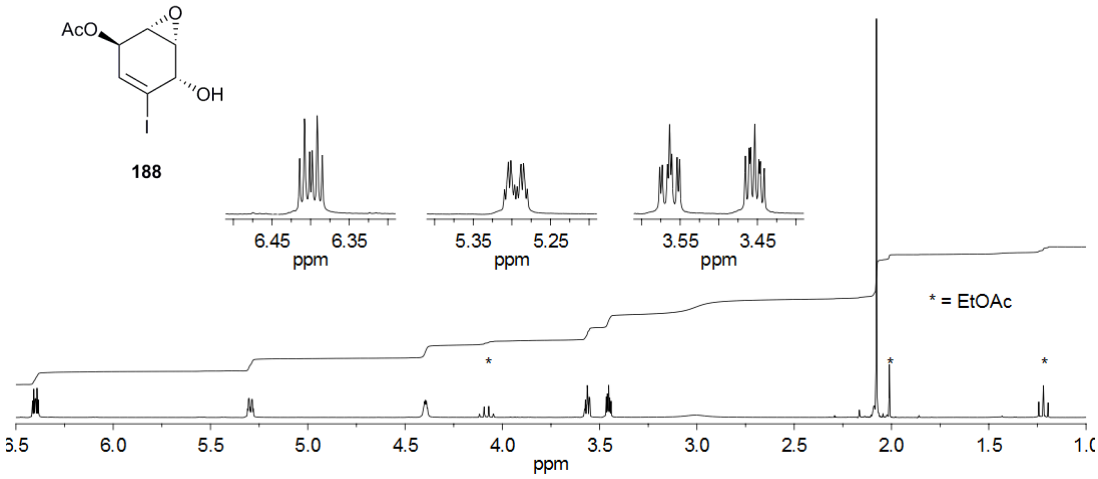


Figure 2-21: *300 MHz ^{1}H NMR spectrum of alcohol **188** (recorded in $CDCl_3$).*
Insets: Expansions of three regions of the spectrum.

The spectral data recorded on ketone **189** bore a strong resemblance to those recorded on congener **103**. ^{1}H NMR spectroscopy indicated that the olefinic proton had become substantially deshielded following the oxidation reaction with the relevant signal appearing as a doublet of doublets at δ 7.36 (Figure 2-22). Furthermore, in the ^{13}C NMR spectrum, the resonance derived from the ketone carbon appears at δ 187.2. No hydroxyl stretching band, so prominent in the precursor, is observed in the IR spectrum of compound **189**. In addition, a new and sharp absorption band at 1695 cm^{-1}, due to ketonic C=O stretching, flanks the ester C=O absorption band at 1743 cm^{-1}. The absorption from olefinic C=C bond stretching was enhanced due to its conjugation with the ketone moiety and appears at 1605 cm^{-1}. The CI mass spectrum of ketone **189** revealed a molecular ion at *m*/*z* 294 and an accurate mass measurement of this species indicated that it possessed the anticipated molecular formula, *viz*. $C_8H_7IO_4$.

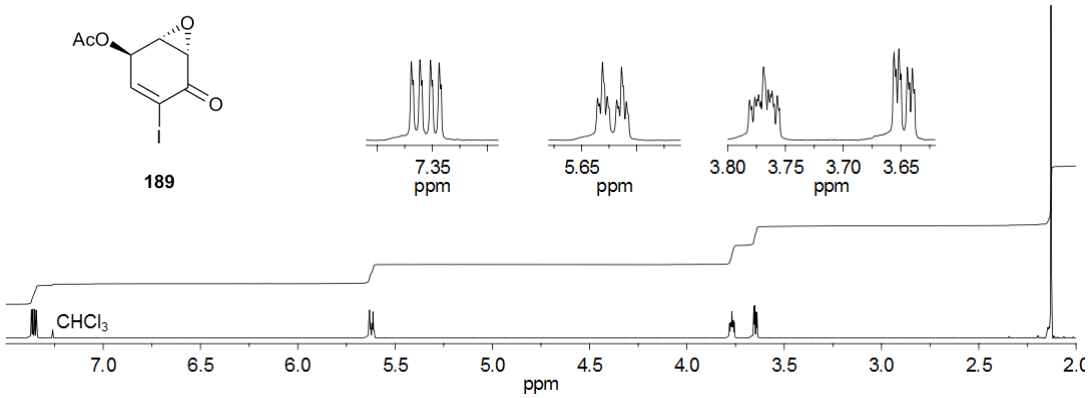
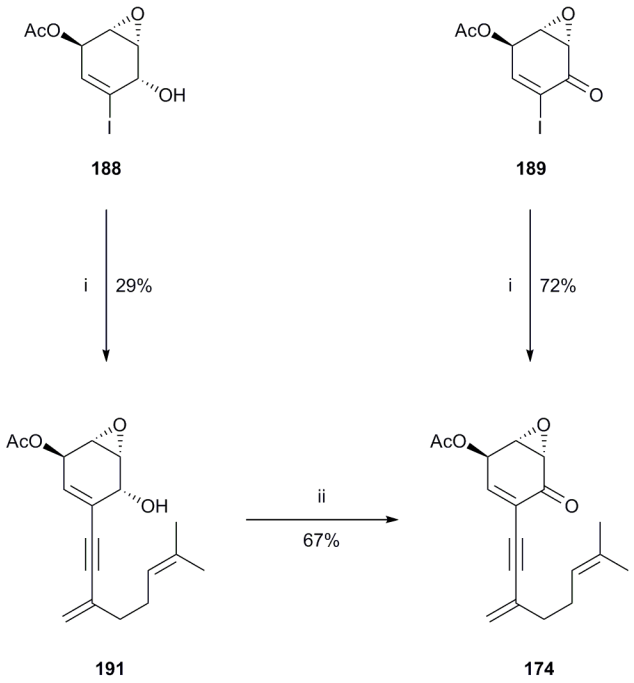


Figure 2-22: *300 MHz ^{1}H NMR spectrum of ketone **189** (recorded in $CDCl_3$).*
Insets: Expansions of three regions of the spectrum.

2.15.3 The Stille cross-coupling reactions

Both iodides **188** and **189** underwent Stille cross-coupling with alkynyl stannane **190** (Scheme 2-43). However, the yield from the first reaction (involving substrate **188**) (29%) was substantially lower than the analogous reaction with its oxidized counterpart, iodide **189** (72%). It is possible that the free hydroxyl group adjacent to the reactive iodide may interfere with the reaction. In the latter case, the reaction produced (−)-tricholomenyn A, in good yield, and completed the shortest preparation of this natural product reported to date.

The spectral data recorded on alcohol **191** were in complete accord with the depicted structure. Despite being obtained in only a modest yield from the Stille cross-coupling reaction, this compound was subsequently oxidized to (−)-tricholomenyn A using pyridinium dichromate and a small amount of acetic acid. Samples of this compound were identical to those prepared *via* the alternate pathway that is also shown in Scheme 2-43.



Scheme 2-43: *Reagents and conditions* (i) compound **190**, Pd[0], Ph_3As, CuI, Et_2O, 20 °C, 1 h; (ii) PDC, AcOH, DCM, 20 °C, 1 h.

The spectral data acquired from synthetic samples of (−)-tricholomenyn A produced from the cross-coupling of ketone **189** with stannane **190**, and *via* the oxidation of alcohol **191**, were identical and in complete agreement with those described in the literature for the natural product.[130] The enonic β-proton of synthetic **174** appeared in the associated ^{1}H NMR spectrum (see Figure 2-23) as a doublet of doublets at δ 6.75. The ^{13}C NMR spectrum exhibited the anticipated 18 signals with the carbonyl carbon resonances appearing at δ 189.5 and 169.6, respectively.

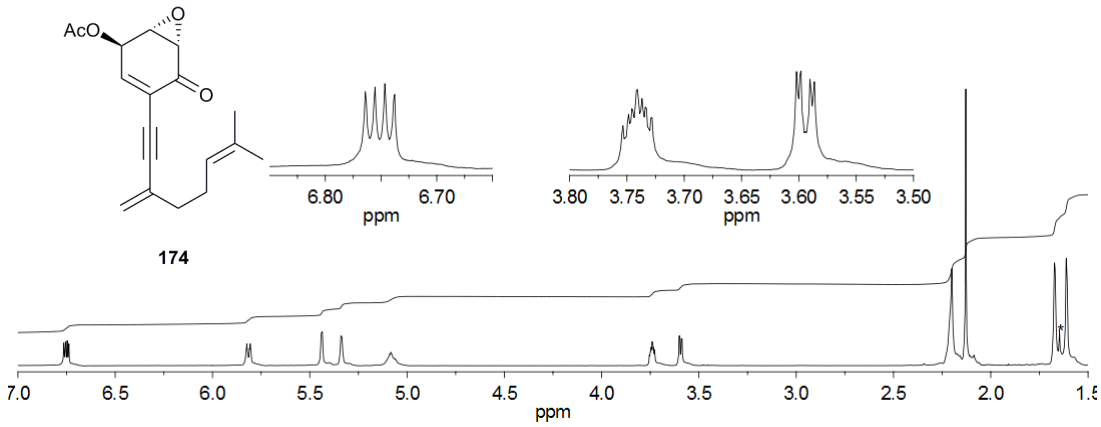


Figure 2-23: *300 MHz ^{1}H NMR spectrum of (−)-tricholomenyn A (recorded in $CDCl_3$).*
Insets: Expansions of two regions of the spectrum.

A comparison of the NMR data recorded on naturally- and synthetically-derived tricholomenyn A shows high levels of consistency (Table 2-7). For instance, the maximum difference between analagous ^{13}C NMR signals is 0.2 ppm and the average difference is just 0.1 ppm. Likewise, ^{1}H NMR shift frequencies vary by no more than 0.05 ppm in any instance and the average difference is 0.02 ppm. As well, coupling constants, where directly comparable, differ by less than 0.5 Hz.

Table 2-7: *Comparison of the ^{13}C and ^{1}H NMR data recorded on naturally- and synthetically-derived tricholomenyn A (***174***)*

^{13}C NMR Data Natural **174** (δ_C) (75 MHz, $CDCl_3$)[130]	^{13}C NMR Data Synthetic **174** (δ_C) (75 MHz, $CDCl_3$)[104]	^{1}H NMR Data Natural **174** (δ_H) (300 MHz, $CDCl_3$)[130]	^{1}H NMR Data Synthetic **174** (δ_H) (300 MHz, $CDCl_3$)[104]
189.3	189.5	6.80 (dd, *J* = 5.0 and 2.5 Hz, 1H)	6.75 (ddd, *J* = 5.4 and 2.4 Hz, 1H)
169.5	169.6	5.83 (dt, *J* = 5.0 and 1.2 Hz, 1H)	5.81 (dt, *J* = 5.4 and 1.2 Hz, 1H)
140.2	140.3	5.45 (d, *J* = 2.0 Hz, 1H)	5.44 (m, 1H)
132.3	132.4	5.35 (d, *J* = 2.0 Hz, 1H)	5.35 (broad s, 1H)
130.4	130.5	5.10 (m, 1H)	5.08 (m, 1H)
124.9	125.0	3.77 (ddd, *J* = 4.0, 2.5 and 1.2 Hz, 1H)	3.74 (m, 1H)
123.4	123.5	3.60 (dd, *J* = 4.0 and 1.2 Hz, 1H)	3.59 (dd, *J* = 3.6 and 1.2 Hz, 1H)
122.9	123.0	2.25 (m, 4H)	2.20 (broad s, 4H)
95.8	95.9	2.14 (s, 3H)	2.14 (s, 3H)
81.8	81.9	1.68 (d, *J* = 0.8 Hz, 3H)	1.68 (s, 3H)
64.0	64.1	1.63 (d, *J* = 1.0 Hz, 3H)	1.62 (s, 3H)
54.6	54.7		
52.8	52.9		
36.8	36.9		
26.5	26.6		
25.6	25.7		
20.5	20.6		
17.6	17.7		

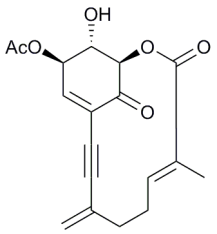
In the IR spectrum of (−)-tricholomenyn A, a weak absorption band observed at 2205 cm^{-1} is indicative of C≡C stretching. The ester moiety gives rise to a C=O absorption band at 1758 cm^{-1} and a C−O stretching band at 1220 cm^{-1}. The EI mass spectrum displayed a molecular ion at *m*/*z* 300 and an accurate mass measurement on this species established that it was of the expected composition, *viz*. $C_{18}H_{20}O_4$.

The total synthesis of (−)-tricholomenyn A (**174**) described here represents the most concise route so far reported. Furthermore, this synthesis matches that of Kamikubo *et al*. in terms of overall yield.[101] A comparison of the key features associated with the six reported syntheses of tricholomenyn A is shown in Table 2-8.

Table 2-8: *A comparison of the key features associated with the six reported syntheses of tricholomenyn A*

Lead author	Publication date	Key bond-forming event	Longest linear sequence	Overall yield (%)	Modification produced
Ogasawara[85]	1996	C6−C1′ (Stille)	twelve steps	18	(−)
Taylor[102]	1997	C6−C1′ (Sonogashira)	eight steps	7	(±)
Johnson[90]	1997	C6−C1′ (Sonogashira)	twelve steps	1	(−)
Negishi[133]	2000	C6−C1′ (Negishi)	ten steps	10	(±)
Lee[92]	2009	C5−C6 (see text)	twelve steps	10	(−)
Present work[104]	2009	C6−C1′ (Stille)	five steps	18	(−)

The highly abbreviated nature of this synthesis lends itself to the synthesis of related of related acetylenic epoxyquinol natural products such as tricholomenyn B (Figure 2-24). Investigations directed towards such ends are now underway in these laboratories.



175

Figure 2-24: *Tricholomenyn B*

2.16 CONCLUSIONS AND FUTURE WORK

The work described here has culminated in the development of chemoemzymatic techniques that allow for the rapid synthesis, at relatively large scale, of many members of the epoxyquinol class of natural product. The key feature of this new route is a three-step and highly selective reaction sequence for the conversion of enantiomerically pure and enzymatically-derived *cis*-1,2-dihydrocatechols into "epoxyquinol building blocks" **101**-**103**. These highly versatile building blocks can be elaborated to some half-dozen epoxyquinol-based natural products. Since the enantiomeric forms of certain of the starting *cis*-1,2-dihydrocatechols are readily available, the optical antipodes of the naturally-occurring epoxyquinols should also be accessible by the methods described here. As such, the development of comprehensive structure-activity relationship (SAR) profiles for this fascinating class of natural products has become a little easier.

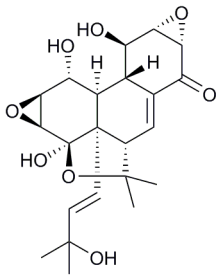
CHAPTER THREE

THE TOTAL SYNTHESES OF DIMERIC EPOXYQUINOL-DERIVED NATURAL PRODUCTS

3.1 (+)-PANEPOPHENANTHRIN

3.1.1 Isolation and characterization of (+)-panepophenanthrin

The dimeric epoxyquinol-derived natural product (+)-panepophenanthrin (Figure 3-1) was isolated by Sekizawa *et al*. during the course of screening the cultured broths of various mushrooms, including *Panus rudis*, for inhibition of an ubiquitin-activating enzyme E1.[134] (+)-Panepophenanthrin (**192**) is the first reported inhibitor of E1 derived from a natural source. The structure of the title compound was initially established through NMR studies and then confirmed by a single-crystal X-ray analysis. Moses and Baldwin proposed that the biosynthesis of (+)-panepophenanthrin may involve the Diels-Alder dimerization of two identical epoxquinol monomers, both of which are, in turn, derived from 4-hydroxybenzoate and demethylallyl pyrophosphate.[96]



192

Figure 3-1: *(+)-panepophenanthrin*

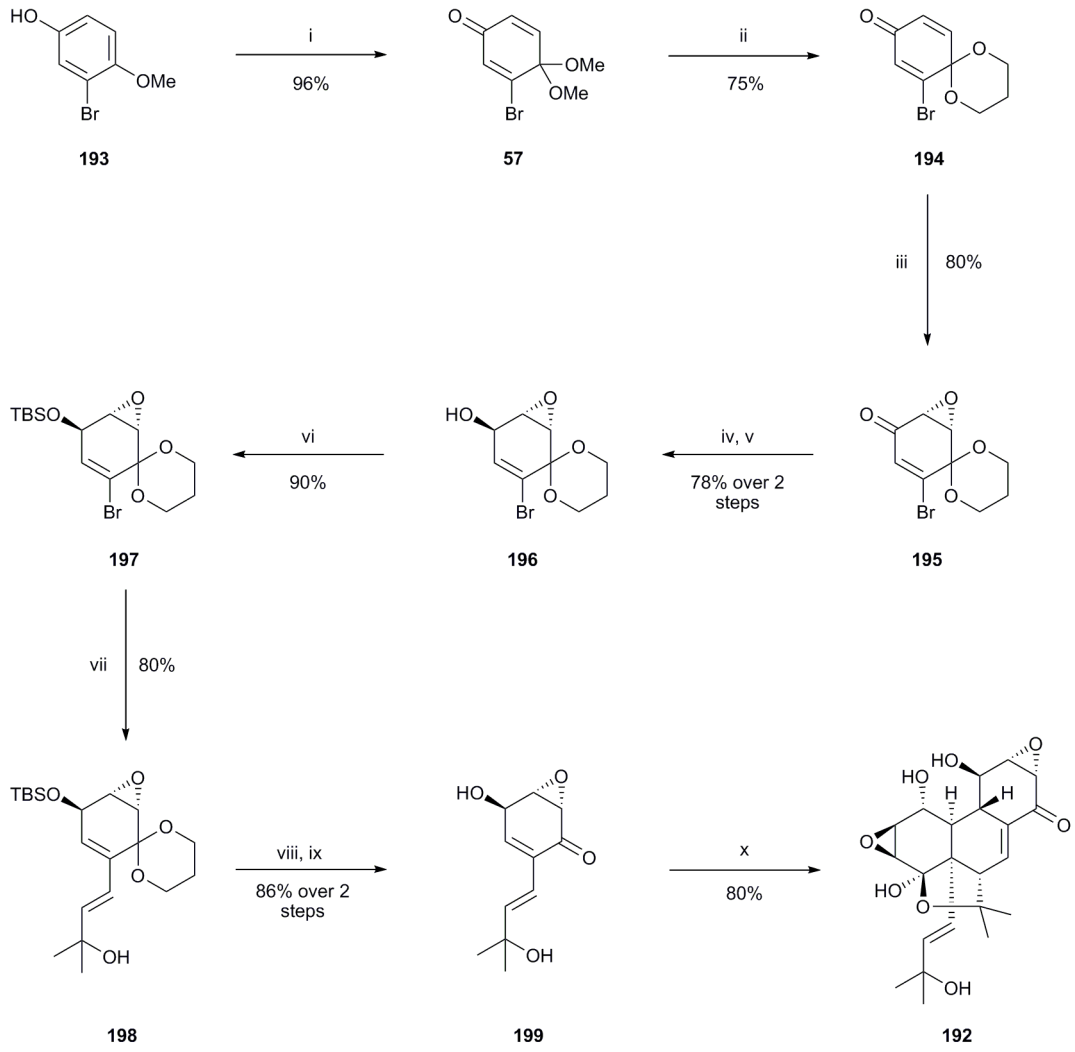
3.1.2 Previous studies on the synthesis of panepophenanthrin

Panepophenanthrin has been the subject of six previous total syntheses.[95-100] All of these involve the preparation of the same epoxyquinol monomer proposed as the biogenetic precursor to the target and which quite clearly engages in the anticipated dimerization process to afford the

polycyclic framework of the natural product. The first synthesis of (+)-panepophenanthrin was reported by Porco *et al.* in 2003 and employed a tartrate-mediated asymmetric epoxidation reaction as a key step.[95] This synthesis was followed, in the same year, by another reported by Baldwin *et al*. which used (±)-bromoxone as a key intermediate in the synthesis of the racemic form of the target.[96] In 2006, the same researchers incorporated an enzymatic resolution step that provided access to (−)-bromoxone and thereby allowing for the preparation of (+)-panepophenanthrin.[99] Mehta *et al*. generated (+)-panepophenanthrin *via* the enzymatic desymmetrization of a diol.[97] Shortly thereafter, this research group produced (−)-panepophenanthrin through a procedure that built upon their earlier synthesis of the natural enantiomer.[98] In 2006, Hayashi described the highly enantioselective α-aminoxylation of a simple ketone and the conversion of the product of this process into (+)-panepophenanthrin.[100] Each of these syntheses is discussed in detail in the following sections.

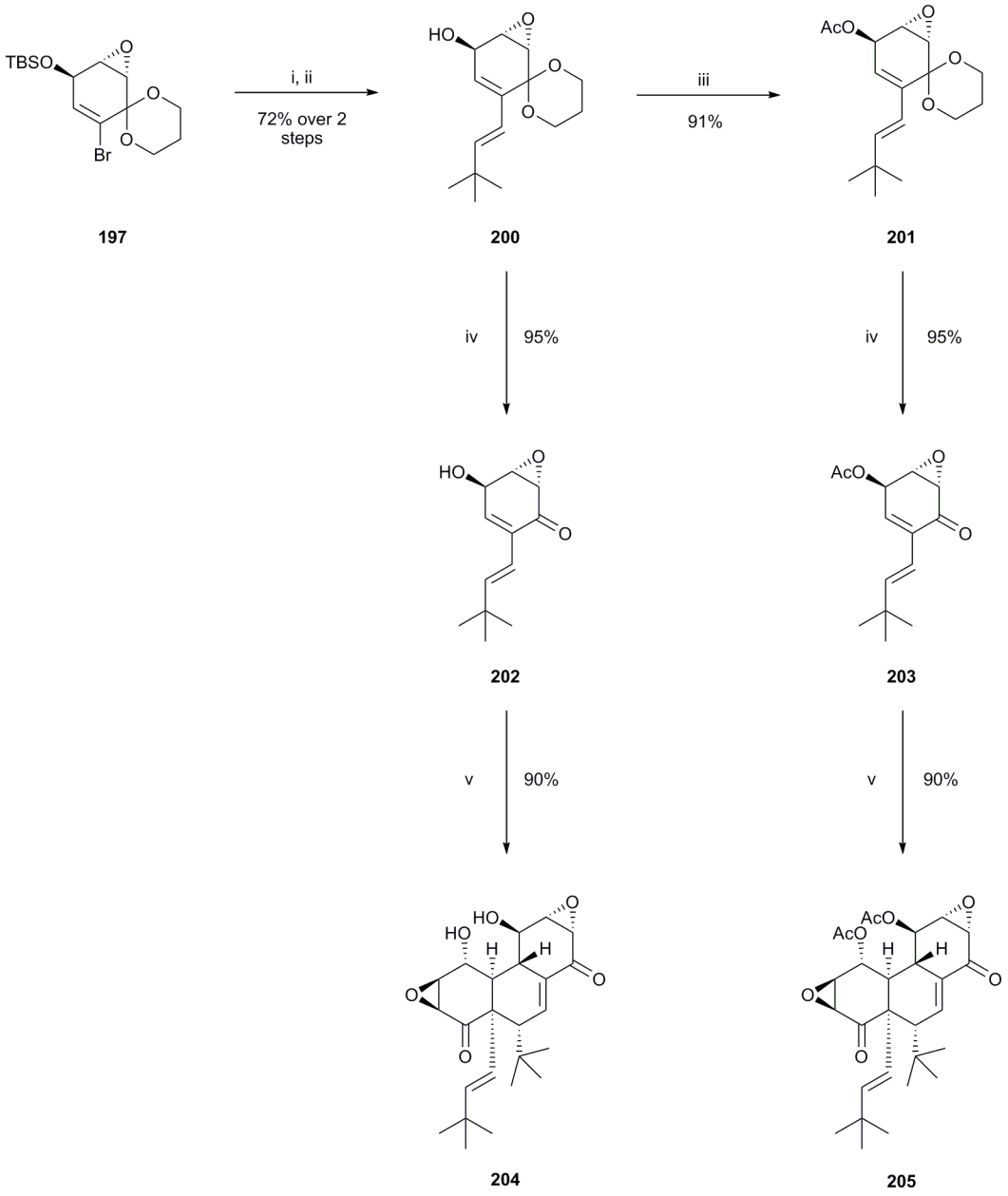
Porco's total synthesis of (+)-panepophenanthrin (2003)

The Porco total synthesis of (+)-panepophenanthrin (**192**) (Scheme 3-1) commenced with the (diacetoxyiodo)benzene-mediated oxidation of hydroquinone mono-methyl ether **193**.[95,135] The ensuing quinone mono-acetal **57** was converted into the corresponding 1,3-dioxane **194**, as the new protecting group incorporated by this means was found to be more stable under conditions employed during the ensuing nucleophilic epoxidation reaction. Thus, (−)-di*iso*propyl tartrate-mediated epoxidation of compound **194** was found to take place selectively with the less-substituted double bond, delivering the depicted enantiomer of keto-epoxide **195** in 95% ee and 80% yield. Lithium triethylborohydride effected reduction of the ketone moiety within compound **195** to deliver the corresponding *syn*-epoxy alcohol. Displacement of the newly introduced hydroxyl moiety in a Mitsunobu reaction and subsequent methanolytic cleavage of the intermediate acetate then furnished the desired compound **196**. This *trans*-epoxy alcohol was protected as the corresponding *tert*-butyldimethylsilyl ether, **197**, which engaged in a Heck cross-coupling reaction with 2-methyl-3-buten-2-ol to afford diene **198**. Exposure of the latter material to aqueous hydrofluoric acid effected both removal of the silyl protecting group and hydrolysis of the ketal. However, a stepwise process involving desilylation of compound **198** with tetra-*n*-butylammonium fluoride and cleavage of the acetal with aqueous hydrochloric acid was found to deliver a higher yield of pivotal diene **199**. Upon storage in the neat state, compound **199** engaged in an inverse electron demand Diels-Alder dimerization reaction and a subsequent lactol-forming process to afford (+)-panepophenanthrin (**192**) in an overall yield of 22% and 10 steps from phenol **193**.



Scheme 3-1: *Reagents* (i) $PhI(OAc)_2$, MeOH; (ii) 1,3-propanediol, $BF_3.OEt_2$; (iii) Ph_3COOH, NaHMDS, (−)-di*iso*propyl tartrate, 4 Å molecular sieves; (iv) $LiEt_3BH$; (v) PPh_3, DIAD, *p*-nitrobenzoic acid; NaOMe, MeOH; (vi) TBSCl, imidazole; (vii) $Pd(OAc)_2$, Ag_2CO_3, 2-methyl-3-buten-2-ol; (viii) TBAF; (ix) H_2O, HCl; (x) neat.

Porco *et al*. considered that in course of the abovementioned synthesis of (+)-panepophenanthrin that an acid-catalyzed ionization of the tertiary alcohol residue of compound **199** might take place and that intermolecular attack of the ensuing carbocation by the lone pair of electrons of a second molecule of ketone **199** would induce the formation of a tethered intermediate, and that the generation of this intermediate may then facilitate a selective and high-yielding Diels-Alder reaction. It was shown, however, that model substrates such as compounds **202** and **203**, which cannot participate in such an ionization process, still undergo the Diels-Alder and lactol forming steps to afford the dimeric systems **204** and **205**, respectively (Scheme 3-2). These results clearly indicate that formation of an ionic intermediate prior to the cycloaddition reaction is not necessary or even likely to be involved in the formation of compound **192**.

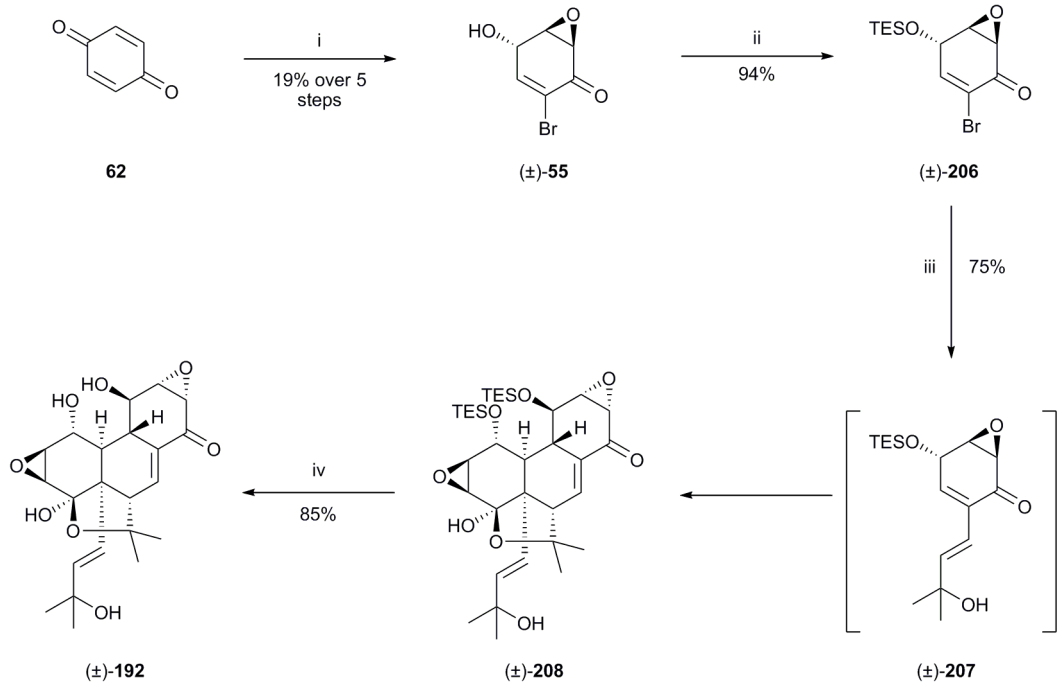


Scheme 3-2: *Reagents* (i) $Pd(OAc)_2$, $AgCO_3$, 3,3-dimethylbutene; (ii) TBAF; (iii) AcOH, 1,3-di*iso*propylcarbodiimide, DMAP; (iv) aqueous HCl; (v) neat.

Baldwin's total synthesis of (±)-panepophenanthrin (2003)

In the Baldwin synthesis of (±)-panepophenanthrin, racemic bromoxone [(±)-**55**], prepared in five steps according to a previously described sequence,[81] served as the starting material in a short total synthesis of the target (Scheme 3-3).[95] These researchers found that it was necessary to protect the free hydroxyl group of bromoxone [(±)-**55**] in order to be able to carry out the necessary Stille cross-coupling reaction of the alkenyl bromide residue with the requisite alkenyl stannane. Thus, a mixture of triethylsilyl chloride and 2,6-lutidine containing a little 4-(*N*,*N*-dimethylamino)pyridine was used to effect the desired protection of compound (±)-**55**. The product from this process, ether (±)-**206**, was then subjected to the depicted Pd[0]-catalyzed

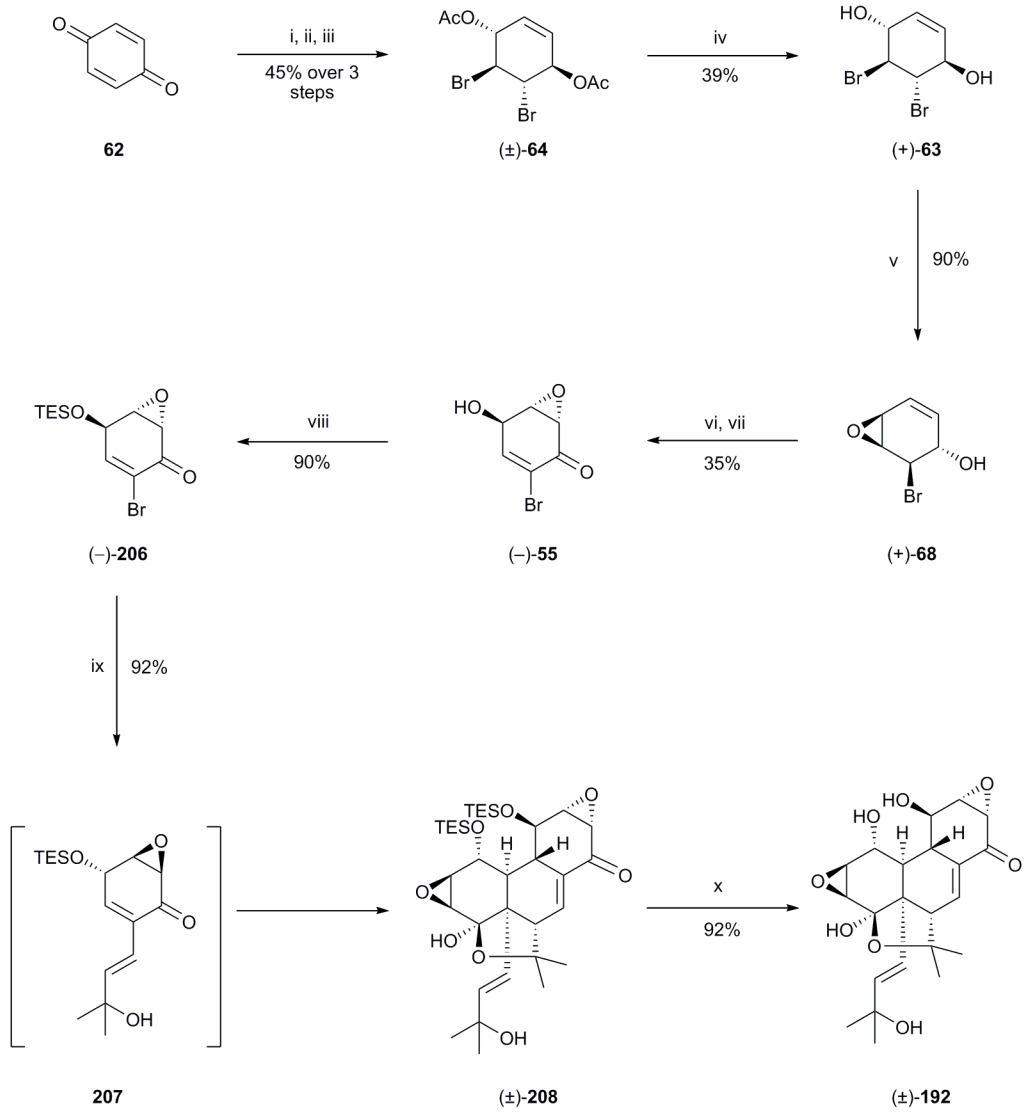
cross-coupling reaction with the indicated alkenyl stannane. From this reaction a mixture of the anticipated product, diene (±)-**207**, and some material arising from Diels-Alder dimerization reaction was isolated. However, due to the propensity for the monomer to readily undergo the Diels-Alder dimerization reaction, full characterization was only carried out on the dimer. In this way, the *bis*-triethylsilyl protected analogue, (±)-**208**, of (±)-panepophenanthrin was obtained in 75% yield from the *O*-triethylsilyl protected (±)-bromoxone [(±)-**207**]. Deprotection of the dimeric material with methanolic ammonium fluoride delivered the target compound (±)-**192** in an overall yield of 11% and in eight steps from *p*-benzoquinone (**62**).



Scheme 3-3: *Reagents* (i) As described by Altenbach *et al*.;[81] (ii) TESCl, 2,6-lutidine, DMAP; (iii) $Pd_2(dba)_3$, $AsPh_3$, (E)-2-methyl-4-(tributylstannyl)but-3-en-2-ol; (iv) NH_4F.

In later work (2006) directed toward the total synthesis of (+)-panepophenanthrin, Baldwin *et al*. used an enzymatic resolution step to obtain (−)-bromoxone and thereby producing the target compound in its naturally occurring enantiomeric form (See Scheme 3-4).[99] Thus, porcine pancreas lipase (PPL) was used to selectively cleave the acetate residues from one enantiomer, (−)-**64**, of a racemic mixture of dibromides. The preparation of compound (±)-**64** from *p*-benzoquinone (**62**) and its elaboration into bromoxone [(−)-**55**] were discussed in detail in Section 2.2.2 within the context of the syntheses of bromoxone reported by Johnson and Altenbach, respectively.[80,81] Protection of the hydroxyl moiety within bromoxone [(−)-**55**] by its reaction with triethylsilyl chloride in the presence of 2,6-lutidine afforded silyl ether (−)-**206**. The application of a reaction sequence parallel to that applied earlier (see above) for the synthesis of (±)-panepophenanthrin, afforded the natural product and with a slightly improved

efficiency over the final two steps. In this way, (+)-panepophenanthrin was obtained in ten steps and with an overall yield of 4% from *p*-benzoquinone **62**.

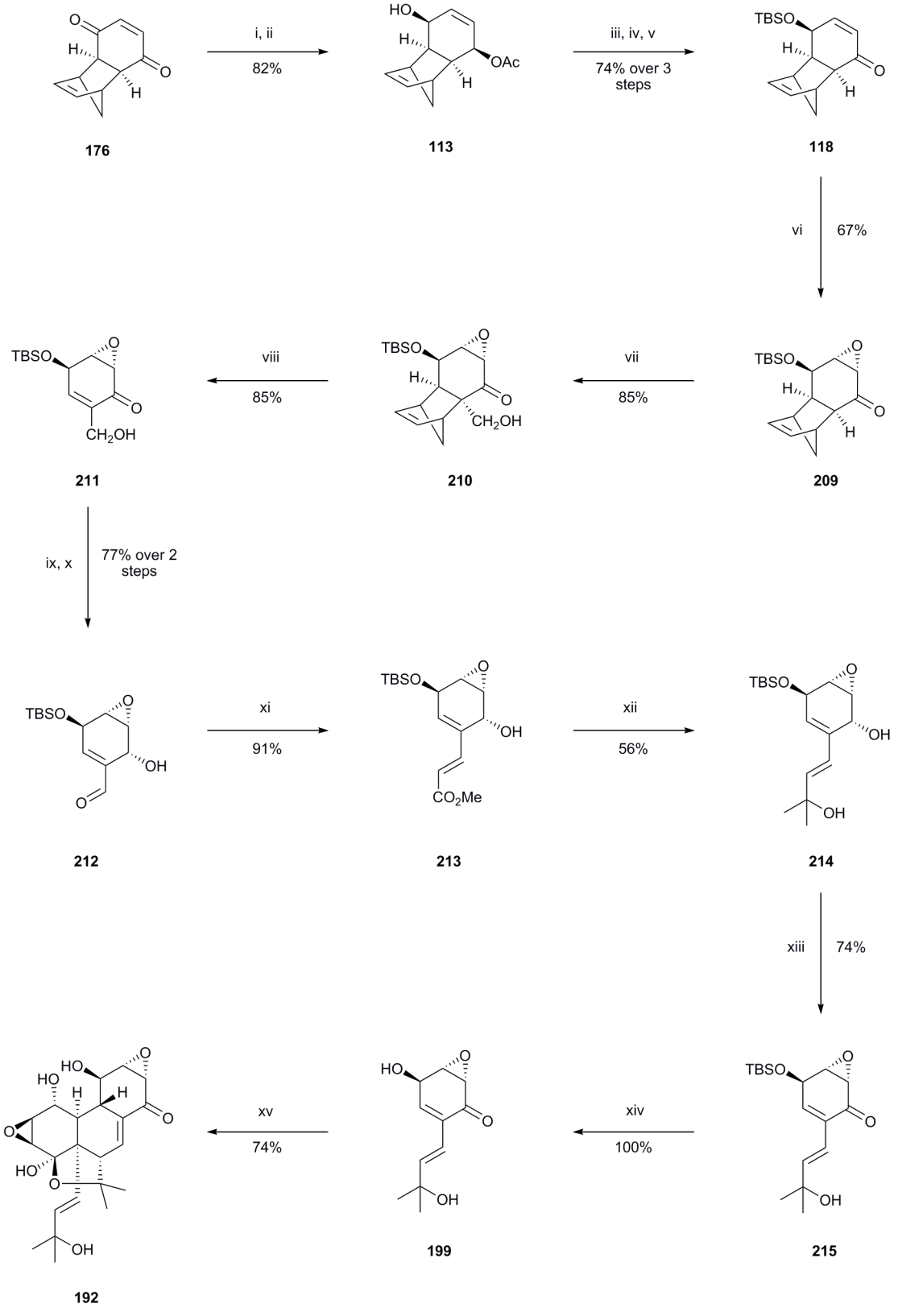


Scheme 3-4: *Reagents* (i) Br_2; (ii) $NaBH_4$; (iii) Ac_2O, Et_3N, DMAP; (iv) PPL, pH 7.0 phosphate buffer; (v) LiOH; (vi) *m*-CPBA; (vii) DMP; $NaHCO_3$, $Na_2S_2O_3$; (viii) TESCl, 2,6-lutidine; (ix) $Pd_2(dba)_3$, $AsPh_3$, (*E*)-2-methyl-4-(tributylstannyl)but-3-en-2-ol.; (x) NH_4F.

Mehta's total synthesis of (+)-panepophenanthrin (2004)

The Diels-Alder adduct, **176**, of *p*-benzoquinone and cyclopentadiene served as the starting material for the total synthesis of (+)-panepophenanthrin reported by Mehta *et al.* in 2004 (Scheme 3-5).[98] Thus, Luche reduction of both ketone carbonyl units within adduct **176** delivered an *endo, endo*-configured *meso*-diol intermediate. Regioselective monoacylation of the ensuing diol, mediated by lipasePS (Amano) and using vinyl acetate as the acyl donor, facilitated the desymmetrization of this intermediate and thereby affording ester **113**. The remaining alcohol residue within compound **113** was *O*-silylated using *tert*-butyldimethylsilyl chloride and subsequent ester group cleavage with basic methanol exposed the other alcohol

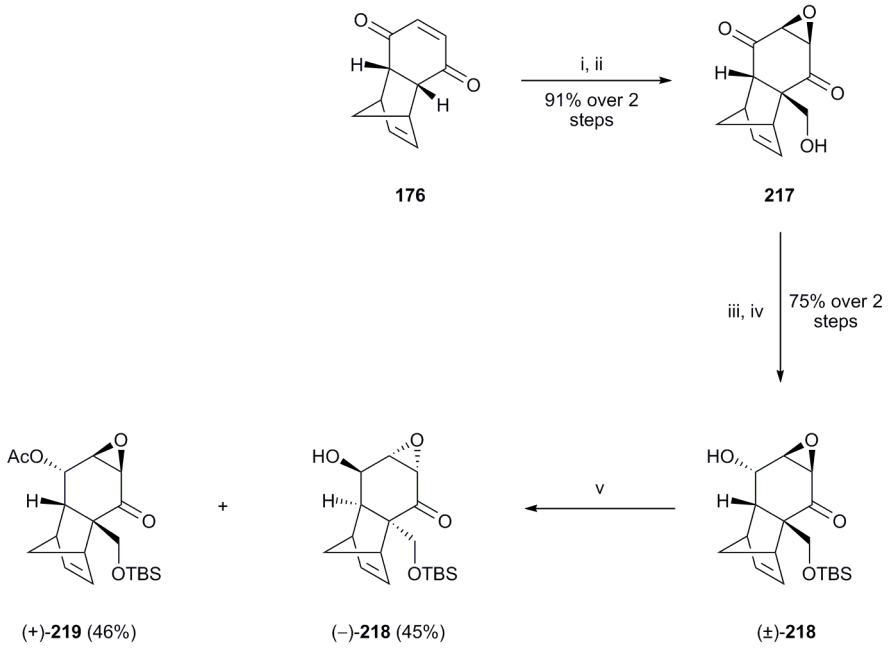
that was oxidized with pyridinium dichromate to afford enone **118**. The newly formed α,β-unsaturated ketone moiety was subjected to nucleophilic epoxidation such that oxygen was delivered to the *exo*-face of the molecule. A catalytic amount of DBU was used to effect an aldol reaction of ketone **209** with formaldehyde and thereby allowing installation of a hydroxymethyl group alpha to the ketone. Compound **210** thus obtained was subjected to a retro-Diels-Alder reaction at 240 °C in diphenyl ether. Di*iso*butylaluminium hydride-mediated reduction of the ensuing enone **211** proceeded diastereoselectively, presumably due to chelation effects, and delivered a *trans*-epoxy alcohol intermediate in a completely diastereoselective manner. The primary alcohol moiety within this intermediate was selectively oxidized with TEMPO, CuCl and oxygen in DMF to the formyl compound **212**. This species was, in turn, homologated using Wittig methodology to deliver the α,β-unsaturated ester **213**. Treatment of ester **213** with an excess of methyl lithium led to the formation of tertiary allylic alcohol **214**. Manganese (IV) oxide was used to oxidize the secondary alcohol moiety within compound **214** to the corresponding ketone **215.** This was followed by a desilylation reaction using the HF/pyridine complex in THF and thus delivering the pivotal monomeric precursor, **199**, to target **192**. Incubation of the monomer, in neat form, at 20 °C for 24 h then gave (+)-panepophenanthrin which was obtained in 15 steps and 6% overall yield from starting material **176**.



Scheme 3-5: *Reagents* (i) $NaBH_4$, $CeCl_3$; (ii) lipasePS (Amano), vinyl acetate; (iii) TBSCl, imidazole, DMAP; (iv) K_2CO_3, MeOH; (v) PDC; (vi) H_2O_2, Na_2CO_3; (vii) DBU, 35% aqueous formalin; (viii) Ph_2O, 240 °C; (ix) di*iso*butyl aluminium hydride; (x) O_2, TEMPO, CuCl; (xi) $Ph_3P{=}CHCO_2Me$; (xii) MeLi; (xiii) MnO_2; (xiv) HF·pyridine; (xv) neat.

Mehta's total synthesis of (−)-panepophenanthrin (2004)

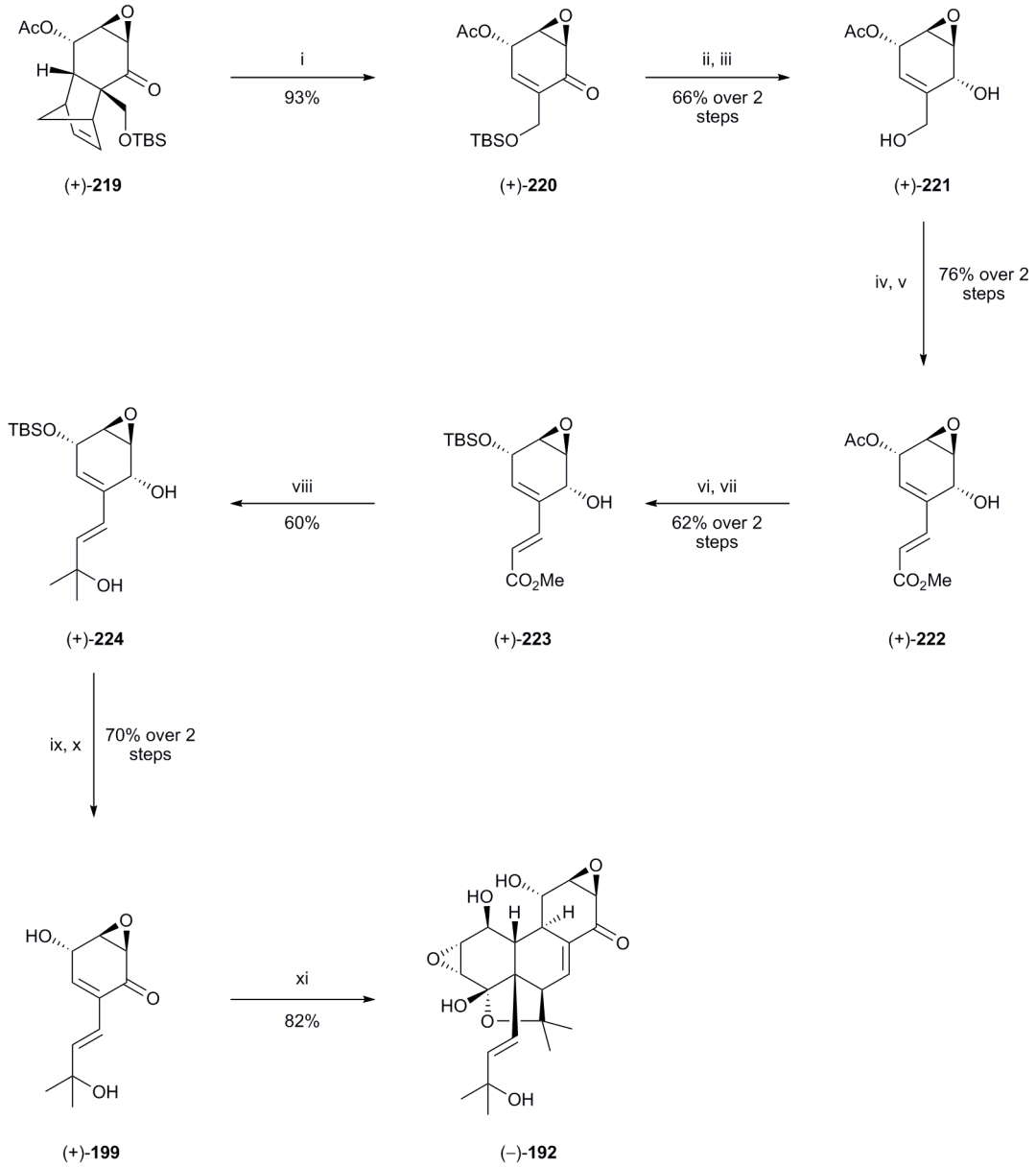
In 2004, Mehta *et al*. synthesized the non-natural or (−)-enantiomeric form, (−)-**192**, of panepophenanthrin (Scheme 3-6). A description of this procedure was published alongside a collection of related syntheses that afforded a number of epoxyquinol and dimeric epoxyquinol-type natural products.[97] Each of these routes proceeded from the Diels-Alder adduct, **176**, of cyclopentadiene and *p*-benzoquinone and involved a late-stage enzymatic resolution step to obtain the desired enantiomeric form of the relevant intermediate. For the synthesis of (−)-panepophenanthrin, nucleophilic epoxidation of alkene **176** was the first step and this proceeded in a stereoselective manner. A catalytic amount of DBU was used to effect an aldol reaction of the ensuing intermediate ketone with formaldehyde and thus allowing installation of a hydroxymethyl group alpha to the ketone thereby delivering the racemic modification of alcohol **217**. This alcohol was protected as the corresponding *tert*-butyldimethylsilyl ether, and sodium borohydride was used to effect reduction of the less sterically hindered ketone and thereby furnishing the *endo*-alcohol (±)-**218**. Enantioselective acetylation of compound (±)-**218**, mediated by lipasePS (Amano) and using vinyl acetate as the acyl donor, faciliated resolution of this racemic mixture. In this way, alcohol (−)-**218** (45% yield, ~99% ee) and ester (+)-**219** (46% yield, ~99% ee) were obtained and the latter could be carried forward to (−)-panepophenanthrin as described immediately below.



Scheme 3-6: *Reagents* (i) 30% aqueous H_2O_2, Na_2CO_3; (ii) DBU, 40% aqueous formalin; (iii) TBSCl, imidazole, DMAP; (iv) $NaBH_4$; (v) Lipase PS-D (Amano), vinyl acetate.

The final reactions employed in Mehta's syntheses of (−)-panepophenanthrin are shown in Scheme 3-7. Thus, a thermally-promoted retro-Diels-Alder reaction allowed for the conversion of compound (+)-**219** into enone (+)-**220**. The silyl protecting group within the latter was then

cleaved with the HF/pyridine complex to afford an intermediate ketone that underwent chelation-controlled reduction with di*iso*butyl aluminium hydride to form diol (+)-**221**. The primary alcohol moiety within product (+)-**221** was selectively oxidized with TEMPO, CuCl and oxygen in DMF to provide the corresponding α,β-unsaturated aldehyde which was reacted with the relevant stabilized ylide to yield the pivotal dienyl ester (+)-**222**. The acetyl protecting group within this ester was exchanged for a *tert*-butyldimethylsilyl ether *via* hydrolysis with lithium hydroxide, in the first instance, and then silylation of the liberated alcohol with the appropriate silyl triflate, in the presence of imidazole and a catalytic quantity of 4-(*N*,*N*-dimethylamino)pyridine. The remaining free alcohol within product (+)-**224** was oxidized to the corresponding ketone using manganese (IV) oxide. The silyl protecting group of the ensuing product was removed with HF-pyridine and thereby afforded the epoxyquinol (+)-**199**. Diels-Alder dimerization of compound (+)-**199** and subsequent lactol formation then took place in a spontaneous fashion and thereby delivering (−)-panepophenanthrin in an overall yield of 3% and in sixteen steps from adduct **176**. This total synthesis is the only one thus far that has provided the unnatural enantiomer of panepophenanthrin.



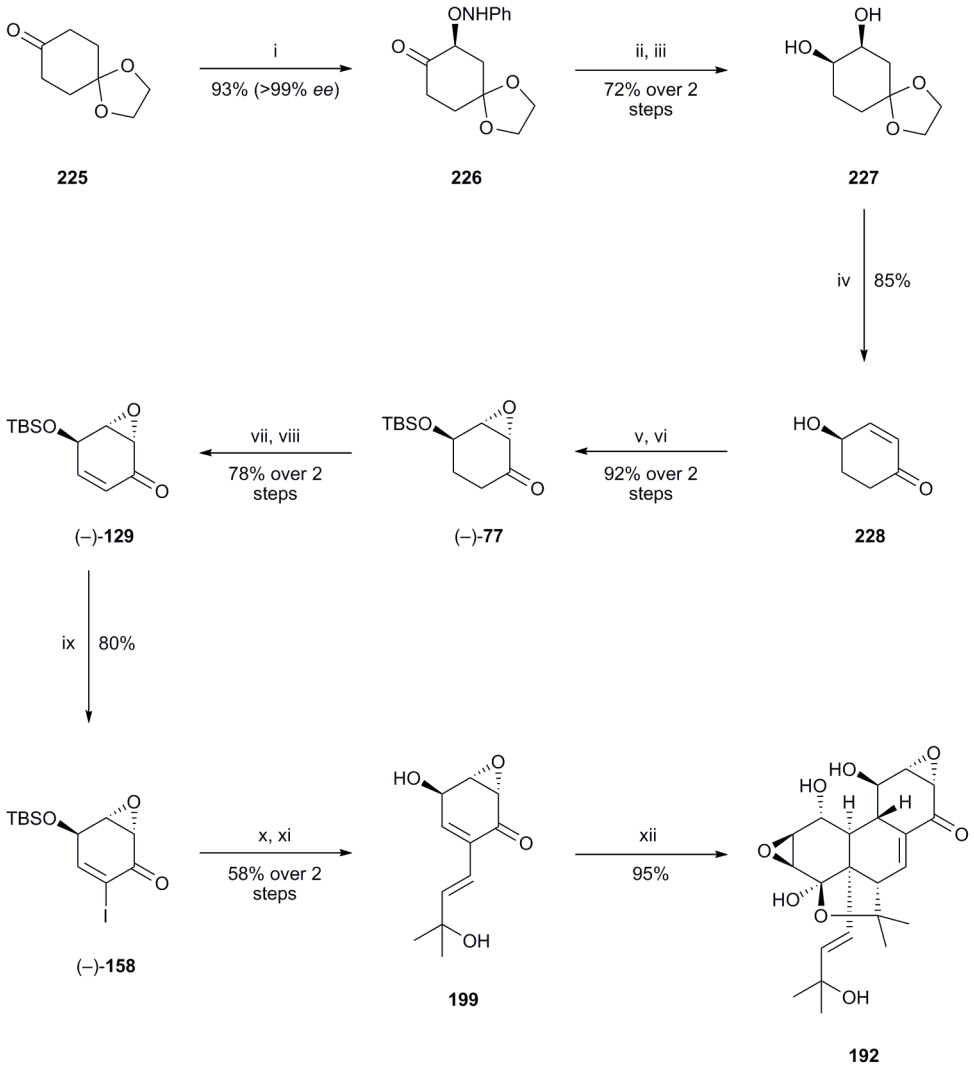
Scheme 3-7: *Reagents* (i) Ph_2O, 240 °C; (ii) HF·pyridine; (iii) di*iso*butylaluminium hydride; (iv) TEMPO, O_2, CuCl; (v) $Ph_3P{=}ChCO_2Me$; (vi) LiOH, MeOH; (vii) TBSOTf, imidazole, DMAP; (viii) MeLi; (ix) MnO_2; (x) HF·pyridine; (xi) neat.

Hayashi's total synthesis of (+)-panepophenanthrin (2006)

In 2006, Hayashi *et al.*[100] reported a total synthesis of (+)-panepophenanthrin wherein the asymmetric α-aminoxylation of 1,4-cyclohexanedione monoethylene ketal (**225**) represented the key and initial step (Scheme 3-8). Thus, 10 mol% (+)-proline was used to catalyze the reaction of nitrosobenzene with substrate **225** and thereby giving compound **226** in greater than 99% enantiomeric excess and in 93% chemical yield. K-selectride-mediated reduction of product **226** was followed by palladium[0]-catalyzed hydrogenolysis of the N-O bond and thereby forming the *cis*-vicinal diol **227**. Treatment of the latter compound with water, in the presence of acid-activated Amberlyst 15 resin, resulted in hydrolysis of the ketal. Dehydration of the resulting β-

hydroxy-ketone then afforded γ-hydroxy-enone **228**. The hydroxyl group within product **228** was protected as the corresponding *tert*-butyldimethylsilyl ether using *tert*-butyldimethylsilyl chloride and imidazole in DMF. The ensuing enone was subjected to nucleophilic epoxidation using hydrogen peroxide and Triton B and thus affording epoxide (−)-**77**. An enone moiety was then installed using the Tsuji-modified Saegusa oxidation procedure and α-iodination of the ensuing enone (−)-**129** was then effected with molecular iodine in the presence of pyridine. Stille cross-coupling of the ensuing iodide (−)-**158** with the requisite alkenyl stannane and deprotection of the resulting silyl ether delivered diol **199**, the by now well-established precursor to (+)-panepophenanthrin. Neat storage of this monomer for 33 h resulted in the anticipated Diels-Alder dimerization reaction and subsequent lactol formation. In this way (+)-panepophenanthrin was obtained in 18% overall yield and in 12 steps from ketone **225**.

As part of their work in this area, Hayashi *et al*. also performed a kinetic study of the Diels-Alder dimerization of compound **199** in a variety of solvents and neat.[100] Unsurprisingly, it was observed that the reaction proceeded most rapidly in the absence of solvent. Furthermore, heavy water was shown to promote a more rapid reaction than either methanol or tetrahydrofuran, supporting the notion that this reaction is biomimetic. In the latter solvents, the reaction ceased at less than 20% conversion after several hours.



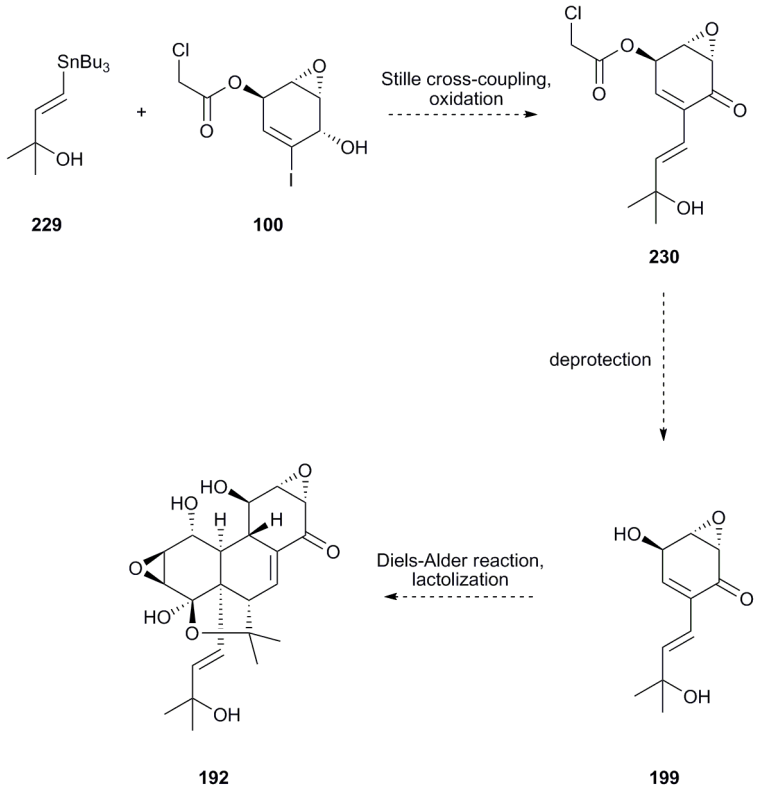
Scheme 3-8: *Reagents* (i) Nitrosobenzene, (+)-proline; (ii) K-selectride; (iii) Pd/C, H_2; (iv) H_2O, amberlyst 15; (v) TBSCl, imidazole; (vi) H_2O_2, triton B; (vii) LHMDS, TMSCl; (viii) diallylcarbonate, $[Pd_2(dba)_3]\cdot CHCl_3$; (ix) I_2, pyridine; (x) $[Pd_2(dba)_3]\cdot CHCl_3$, $AsPh_3$, (*E*)-2-methyl-4-(tributylstannyl)but-3-en-2-ol; (xi) NH_4F; (xii) neat.

3.2 SYNTHETIC STRATEGY ASSOCIATED WITH THE PRESENT SYNTHESIS OF (+)-PANEPOPHENANTHRIN

3.2.1 Proposed synthetic route to (+)-panepophenanthrin

In the present work, a sequence of reactions involving Stille cross-coupling of stannane **229** and iodide **100**, followed by oxidation of the allylic alcohol moiety within the product, was expected to deliver enone **230**. Removal of the α-chloroacetyl protecting group from product **230** would then provide epoxyquinol monomer **199**. At this point, the total synthesis of (+)-panepophenanthrin would be secure as the transformation of compound **199** into the target is well established. This approach has the advantage that intermediate **100** can be readily obtained in just three steps and in enantiomerically pure form from the abundant *cis*-

dihydrocatechol, **90** as described in Section 2.3. The successful application of this synthetic route to (+)-panepophenanthrin is described in the following section.



Scheme 3-9: *The planned synthesis of (+)-panepophenanthrin*

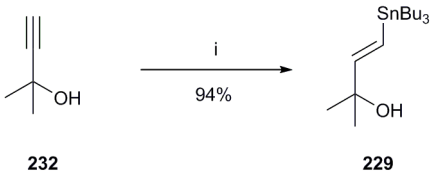
3.3 TOTAL SYNTHESIS OF (+)-PANEPOPHENANTHRIN

Though iodide **100** is available in just three steps and in enantiomerically pure form from the abundant *cis*-dihydrocatechol **90**, it was necessary to prepare stannane **229** in order to carry out the proposed Stille cross-coupling reaction. Efforts to do so are described immediately below and followed by a discussion of the Stille cross-coupling and subsequent reactions *en route* to (+)-panepophenanthrin.

3.3.1 Preparation of the *trans*-alkenyl stannane

Target stannane **229** was readily obtained, as a clear, colorless oil, after chromatography and in 94% yield, *via* Pd[0]-catalyzed hydrostannylation of the commercially available propargyl alcohol **232** with tri-*n*-butyl hydride (Scheme 3-10).[136] According to literature descriptions of this process, the tin radical formed *in situ* adds to the unsubstituted terminus of alkyne **232** and thereby providing the more substituted vinyl radical. In the second step of the process, addition of a hydrogen atom then takes place on the less substituted side of this radical thus producing the addition product and a tri-*n*-butyl tin radical and thereby allowing the radical chain reaction to continue. While the kinetic product of this process is the *Z*-isomer, clean isomerization of this

compound, *via* a radical pathway, affords the more thermodynamically stable *E*-isomer **229** as the only isolable product of the reaction.



232 **229**

Scheme 3-10: *Reagents and conditions* (i) *n*-Bu_3SnH, Pd[0], THF, 20 °C, 1 h.

The ^{13}C NMR spectrum recorded on stannane **229** is shown in Figure 3-2 and, in accord with the depicted structure, eight distinct carbon signals are observed. The signals due to the sp^2-hybridized carbons are observed at δ 155.5 and 122.3 as compared with shifts of δ 89.0 and 70.2 for the analogous carbons in the alkynic precursor. As expected for a stannane such as **229**, the ^{13}C NMR spectrum exhibits certain carbon to tin couplings. From the butyl residues, those ^{13}C nuclei one to three bonds removed from ^{117}Sn or ^{119}Sn display through-bond coupling, with magnitudes ranging from 682.8 Hz (for one bond coupling) to 40.8 Hz (for three bond coupling). In addition, the sp^2-hybridized ^{13}C nuclei adjacent to ^{117}Sn or ^{119}Sn exhibit coupling constants of 752.6 and 719.4 Hz, respectively. The IR spectrum recorded on this sample shows the anticipated hydroxyl-stretching band at 3364 cm^{-1}. The ESI mass spectrum displays an $[M+H]^+$ ion at *m/z* 375 and accurate mass measurement of this species established that it was of the expected composition, *viz*. $C_{17}H_{36}O^{120}Sn$.

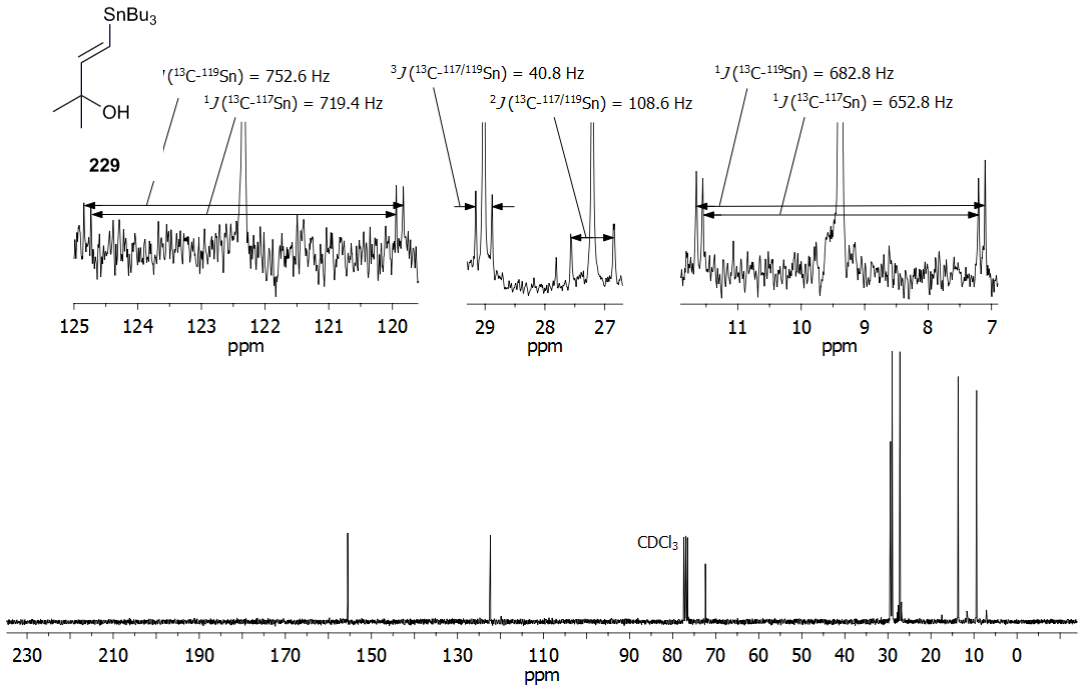
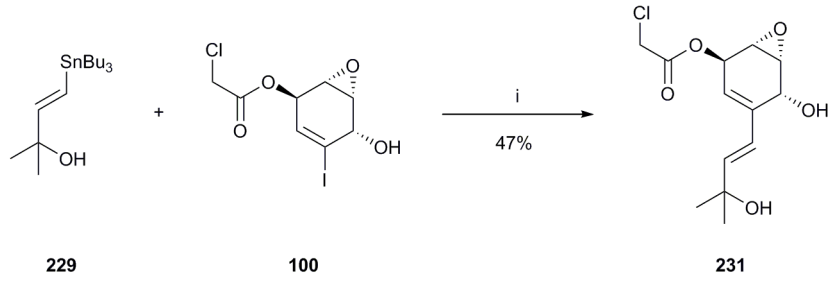


Figure 3-2: *300 MHz ^{13}C NMR spectrum of alkenyl stannane* ***229*** *(recorded in $CDCl_3$).*
Insets: Expansions of three regions of the spectrum showing couplings between ^{13}C and $^{117/119}Sn$ nuclei.

3.3.2 The Stille cross-coupling reaction

Stille cross coupling of compound **229** with iodide **100** (Scheme 3-11) proceeded under the same conditions as those employed earlier for the synthesis of (−)-harveynone (Section 2.15.3). The product diol, **231**, was thereby obtained in 47% yield.



229 **100** **231**

Scheme 3-11: *Reagents and conditions* (i) Pd[0], Ph_3As, CuI, acetone, 50 °C, µwave, 1 h.

The 300 MHz ^1H NMR spectrum of compound **231** (Figure 3-3) displays a pair of mutually coupled doublets arising from the olefinic protons of the pendant sidechain, and these have a coupling constant of 16.2 Hz. This indicates that the *trans*-geometry of the coupling partner **229** has been maintained in the course of the cross-coupling process.

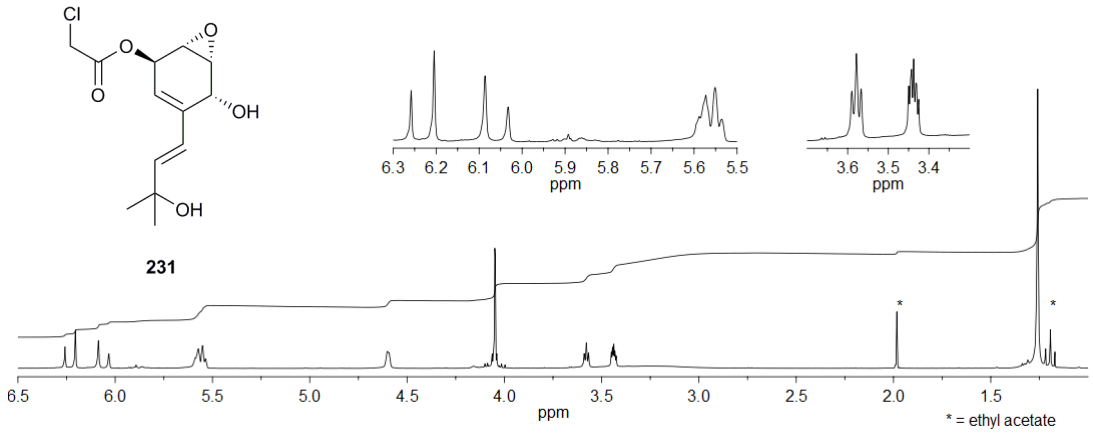
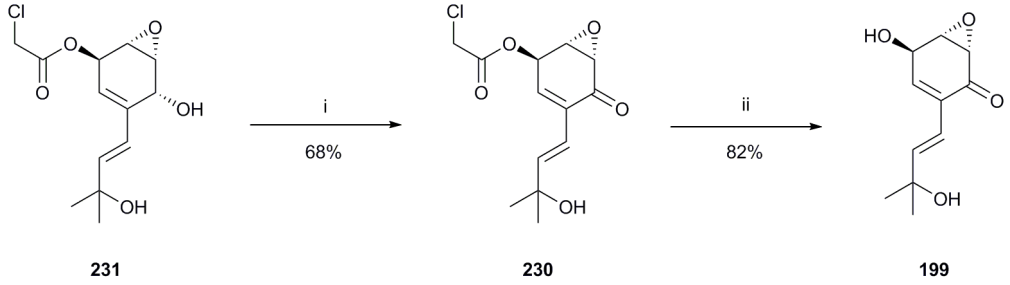


Figure 3-3: *300 MHz ^{1}H NMR spectrum of diene **231** (recorded in $CDCl_3$).*
Insets: Expansions of two regions of the spectrum.

3.3.3 Alcohol oxidation and ester cleavage

In accord with the planned synthetic scheme, alcohol **231**, obtained as described above, was subjected to oxidation with PDC in the presence of acetic acid to give enone **230** in 68% yield (Scheme 3-12). Treatment of the product with zinc acetate in methanol at 20 °C for 1 h then gave the epoxyquinol monomer **199** in 82% yield.



Scheme 3-12: *Reagents and conditions* (i) PDC, AcOH, DCM, 20 °C, 5 h; (ii) $Zn(OAc)_2.2H_2O$, MeOH, 20 °C, 1 h.

The ^{1}H NMR spectrum recorded on monomer **199**, and shown in Figure 3-4, is in agreement with that reported by Porco *et al*. for the same compound.[95] Thus, the C5 proton resonated as a doublet of doublets at δ 6.57 due to its coupling to the protons at C3 and C4. The magnitude of the coupling (J = 16.2 Hz) between the pair of mutually coupled doublets arising from the protons on the *exo*-cyclic alkene clearly indicates that the olefin possesses the *E*-configuration. The geminally-related methyl groups of the sidechain are diastereotopic and appear as a pair of singlets at δ 1.34 and 1.32.

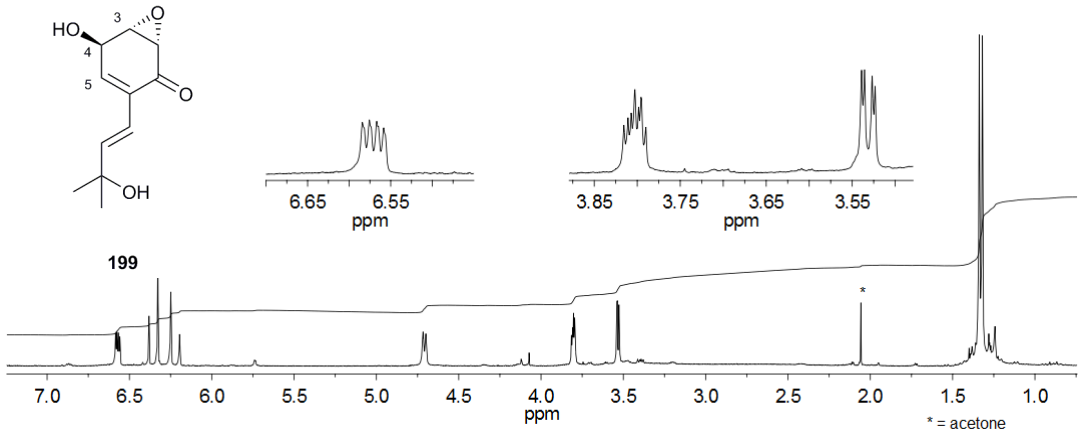
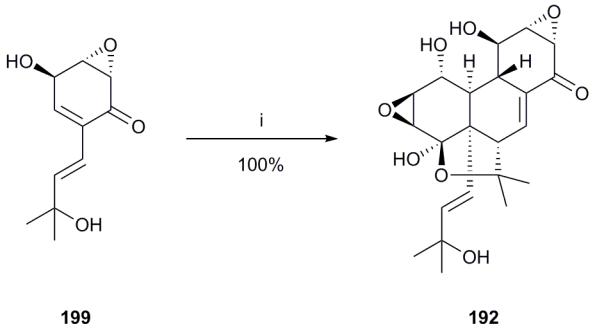


Figure 3-4: *300 MHz ^{1}H NMR spectrum of alcohol **199** (recorded in $CDCl_3$).*
Inset: Expansions of two regions from the spectrum.

Hydroxyl stretching results in a broad absorption band centred at 3411 cm^{-1} in the IR spectrum of alcohol **199**. The conjugated enone moiety gives rise to a strong C=O absorption band at 1683 cm^{-1} while the EI mass spectrum displays an ion at *m/z* 195 that derives from the loss of a methyl group from the parent ion. An accurate mass measurement on this species established that it was of the expected composition, *viz.* $C_{10}H_{11}O_4$. Since epoxyquinol product **199** is a known precursor to (+)-panepophenanthrin, the synthesis of the natural product was formally established at this point.

3.3.4 The Diels-Alder dimerization reaction

While compound **199** could be subjected to full spectroscopic characterization, it displayed a propensity to undergo dimerization to give (+)-panepophenanthrin (**192**). Thus, on standing at *ca*. 20 °C for 72 h, neat samples of compound **199** were converted into the dimer **192** *via* a [4 + 2] cycloaddition reaction and a subsequent lactol forming reaction (Scheme 3-13). The product was obtained in a quantitative yield and as a crystalline solid with a melting point of 140−144 °C. This compared favourably with the reported value, *viz*. 145−148 °C.[134] The optical rotation recorded on this material is $[\alpha]_D$ +144 (*c* 0.5, methanol) and was, therefore, of similar magnitude to that reported for the natural product {$[\alpha]_D$ +146 (*c* 1.0, methanol)}.[134]



199 **192**

Scheme 3-13: *Reagents and conditions* (i) neat, 20 °C, 72 h.

The ^{1}H NMR spectrum of (+)-panepophenanthrin (**192**) is shown in Figure 3-5. The chemical shifts for signals appearing in this spectrum, as well as the remaining spectroscopic data, are in good agreement with those published earlier for the target compound.[95-100] A comparison of the ^{13}C and ^{1}H NMR data recorded on naturally- and synthetically-derived (+)-panepophenanthrin (**192**) is shown in Table 3-1.

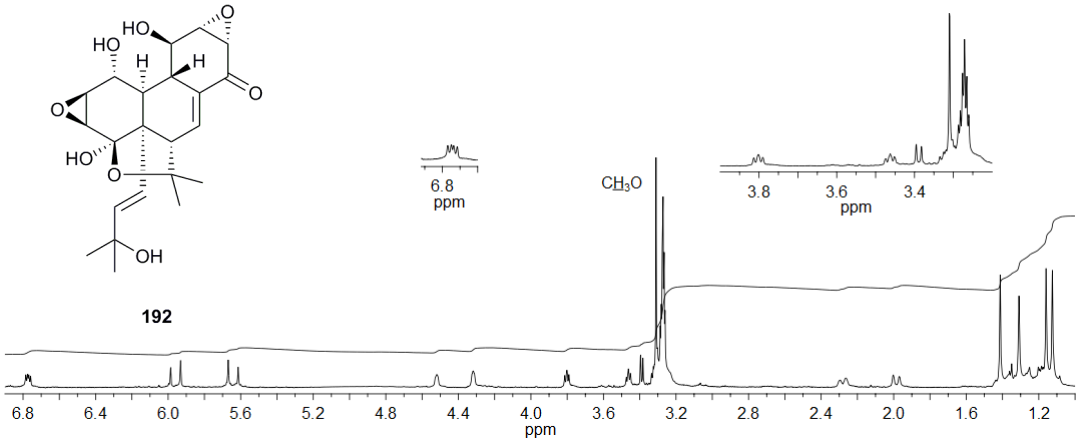


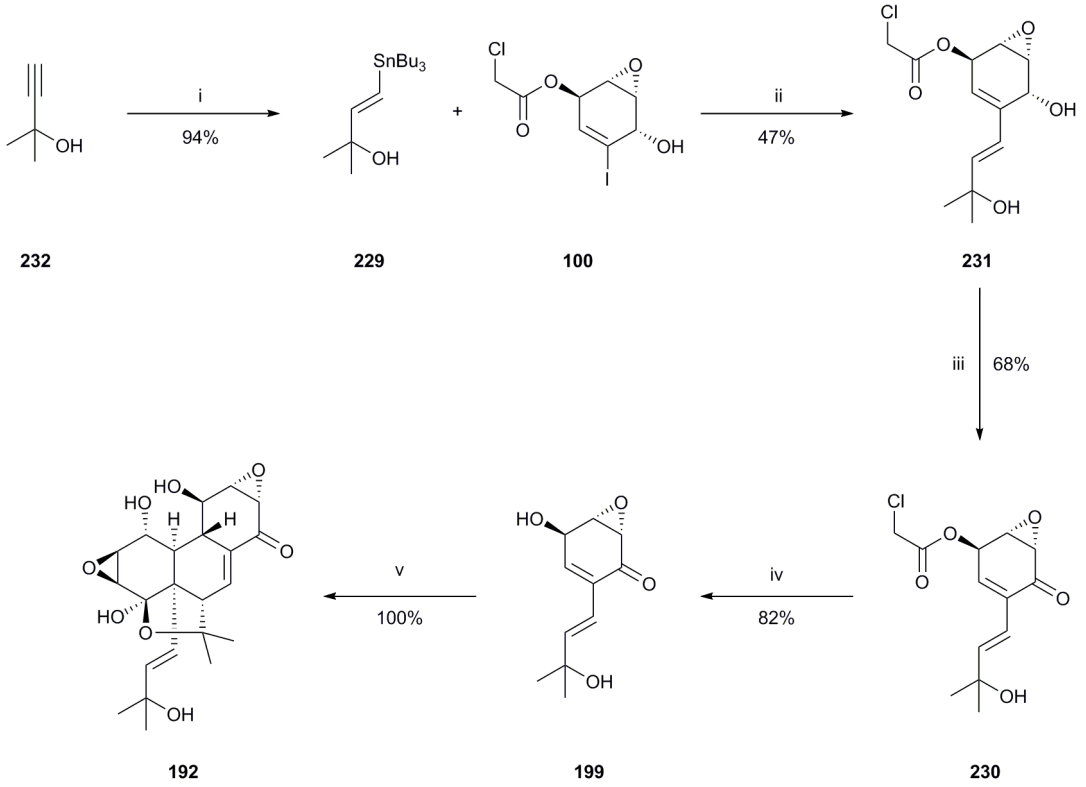
Figure 3-5: *300 MHz ^{1}H NMR spectrum of (+)-panepophenanthrin (recorded in d_4-methanol). Inset: Expansions of two regions from the spectrum.*

It can be seen in Table 3-1 that the ^{13}C NMR spectrum recorded on the synthetically-derived compound closely matches that recorded on the natural product. The Δδ values of the relevant signals are less than 0.2 ppm. The ^{1}H NMR spectral data are equally well-matched with Δδ values for the relevant signals being 0.05 ppm or less.

Table 3-1: *Comparison of the ^{13}C and ^{1}H NMR data recorded on naturally- and synthetically-derived (+)-panepophenanthrin (***192***)*

^{13}C NMR Data Natural **192** (δ_C) (75 MHz, $CDCl_3$)[134]	^{13}C NMR Data Synthetic **192** (δ_C) (75 MHz, $CDCl_3$)	^{1}H NMR Data Natural **192** (δ_H) (300 MHz, $CDCl_3$)[134]	^{1}H NMR Data Synthetic **192** (δ_H) (300 MHz, $CDCl_3$)
196.2	196.2	6.81 (dd, *J* = 5.0 and 3.0 Hz, 1H)	6.77 (dd, *J* = 4.5 and 2.7 Hz, 1H)
143.0	142.9	5.99 (d, *J* = 16.0 Hz, 1H)	5.96 (d, *J* = 16.5 Hz, 1H)
139.9	140.0	5.68 (d, *J* = 16.0 Hz, 1H)	5.64 (d, *J* = 16.5 Hz, 1H)
138.8	138.8	4.55 (broad dd, *J* = 2.0 Hz, 1H)	4.52 (broad s, 1H)
129.2	129.3	4.35 (broad t, *J* = 2.0 Hz, 1H)	4.32 (broad s, 1H)
102.6	102.7	3.84 (broad t, *J* = 4.0 Hz, 1H)	3.80 (t, *J* = 3.6 Hz, 1H)
79.2	79.1	3.50 (broad t, *J* = 4.0 Hz, 1H)	3.46(broad t, *J* = 3.6 Hz, 1H)
71.8	71.8	3.42 (d, *J* = 4.0 Hz, 1H)	3.39 (d, *J* = 4.2 Hz, 1H)
69.0	69.0	3.35 (dd, *J* = 5.0 and 2.0 Hz, 1H)	3.28-3.26 (m, 2H)
66.2	66.2	3.32 (d, *J* = 4.0 Hz, 1H)	
60.7	60.6	2.33 (m, 1H)	2.28 (d, *J* = 9.3 Hz, 1H)
57.3	57.4	2.03 (broad d, *J* = 10.0 Hz, 1H)	1.99 (d, *J* = 9.3 Hz, 1H)
57.2	57.2	1.45 (s, 3H)	1.41 (s, 3H)
57.1	57.1	1.35 (s, 3H)	1.31 (s, 3H)
55.6	55.6	1.20 (s, 3H)	1.16 (s, 3H)
55.1	55.1	1.17 (s, 3H)	1.13 (s, 3H)
51.2	51.2		
50.0	49.9		
32.3	32.3		
30.3	30.3		
29.4	29.5		
26.1	26.2		

Scheme 3-14 provides an overview of the reaction sequence leading to the total synthesis of (+)-panepophenanthrin from iodide **100**. As iodide **100** is readily obtained from *cis*-dihydrocatechol **90** in just three steps (see Section 2.3), this synthesis delivered the target in an overall yield of 11% and in just seven steps from the commercially available *cis*-dihydrocatechol **90**.



Scheme 3-14: *Reagents and conditions* (i) *n*-Bu_3SnH, Pd[0], THF, 20 °C, 1 h; (ii) Pd[0], Ph_3As, CuI, acetone, 50 °C, μwave, 0.3 h; (iii) PDC, acetic acid, dichloromethane, 20 °C, 1h; (iv) $Zn(OAc)_2.2H_2O$, MeOH, 20 °C, 1 h; (v) neat, 20 °C, 72 h.

As Table 3-3 indicates, the new synthetic route described in this work provides the shortest linear sequence for the preparation of panepophenanthrin. This route also avoids the use of silicon-based protecting groups and the associated cleavage of these groups with hazardous fluoride-based reagents.

Table 3-2: *A comparison of the key features associated with the seven reported syntheses of panepophenanthrin*

Lead author	Publication date	Longest linear sequence	Overall yield (%)	Modification produced
Porco[93]	2003	ten steps	22	(+)
Baldwin[96]	2003	ten steps	4	(±)
Mehta[98]	2004	fifteen steps	6	(+)
Mehta[97]	2004	sixteen steps	3	(−)
Hayashi[134]	2006	twelve steps	18	(+)
Present work[104]	2009	seven steps	11	(+)
Lee[137]	2010	twenty steps	2	(+)

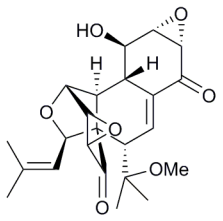
Following the publication of the present work, Lee and Li reported their total synthesis of (+)-panepophenanthrin.[137] This route made use of the enyne metathesis and [1,3]-metallotropic shift methodology that was used by the same researchers in earlier syntheses of (−)-harveynone and (−)-tricoholomenyn A (see Sections 2.10.2 and 2.13.2, respectively).[92] Although this

synthesis was lengthy and relatively low-yielding, it did demonstrate that a relay metathesis-based tandem ring-closing metathesis and cross metathesis approach is an alternative to Pd[0]-catalyzed cross-coupling reactions for the construction of the scaffold of epoxyquinol-based natural products.

3.4 (+)-HEXACYCLINOL

3.4.1 Isolation and characterization of (+)-hexacyclinol

In an effort to identify new bioactive metabolites, (+)-hexacyclinol (**233**) (Figure 3-6) was isolated by Gräfe *et al*. from a cultured fungus, identified as *Panus rudis* HKI 0254, that had been collected from fallen timber near Irkutsk in Siberia.[138] A study of the biological activity of this isolate revealed that it possessed some antiproliferative properties and displayed moderate inhibitory activity against *Plasmodium falciparum*, the protozoan parasite responsible for the most dangerous form of malaria in humans. An unusual oligocyclic structure containing epoxide and endoperoxide groups was initially assigned to this compound and a total synthesis of this structure was claimed by La Clair but has since been shown to be bogus.[139] The correct structure for (+)-hexacyclinol, *viz*. the dimeric epoxyquinol system **233**, was proposed by Rychnovsky with the aid of computational methods. Two subsequent and related computational studies supported this reassignment.[140,141] Computationally-predicted ^{13}C shift values for this structure correlated well with experimental data collected on other structurally related and highly oxygenated natural products. It was initially considered that (+)-hexacyclinol was produced by *via* a silica gel-mediated rearrangement of the structurally-related natural product (+)-panepophenanthrin.[93] As this proposal was disproven, it is now expected that the biosynthesis of (+)-hexacyclinol is similar to that of (+)-panepophenanthrin and that both of these natural products are derived from the Diels-Alder dimerization of the corresponding epoxyquinol monomers.[94]



233

Figure 3-6: *(+)-Hexacyclinol.*

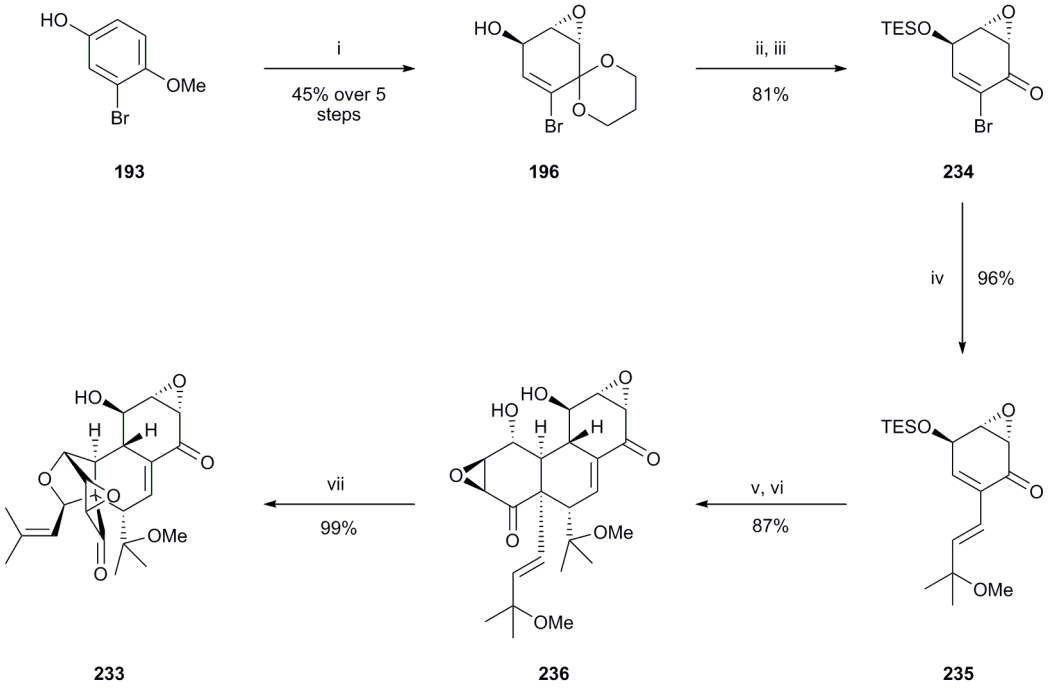
3.4.2 Previous studies on the synthesis of hexacyclinol

Hexacyclinol has been the subject of two previous total syntheses.[93,94] Both of these involve the preparation of the same epoxyquinol monomer proposed as the biogenetic precursor to the

target and which quite clearly engages in the anticipated dimerization process *en route* to the natural product.

Porco's total synthesis of (+)-hexacyclinol (2006)

Since it was considered possible that (+)-hexacyclinol may have been produced *via* a silica gel-mediated rearrangement of (+)-panepophenanthrin in the presence of methanol, Porco *et al.* exposed synthetically-derived (+)-panepophenanthrin to a range of acids in the presence of methanol. However, these efforts failed to effect the desired outcome. Subsequently, the same authors developed a total synthesis of (+)-hexacyclinol that proceeded from enantiopure epoxy alcohol **196** (Scheme 3-15).[93] Alcohol **196** was obtained from hydroquinone mono-methyl ether **193** in five steps including an asymmetric epoxidation reaction mediated by (−)-tartaric acid.[135] This epoxyalcohol was utilized in an earlier synthesis of (+)-panepophenanthrin.[95] From compound **196**, an α,β-unsaturated ketone was revealed by hydrolysis with K10 clay in dichloromethane, and the liberated hydroxyl group was protected to afford triethylsilyl ether **234**. Stille cross-coupling of this compound with the relevant alkenyl stannane took place at 100 °C over one hour and thereby afforded dienyl ether **235**. The triethylsilyl protecting group within compound **235** was cleaved with the hydrofluoric acid/triethylamine complex and the ensuing alcohol was incubated, neat, for 72 h. This facilitated an *exo*-Diels-Alder dimerization reaction, that is probably biomimetic, and that afforded (+)-pre-hexacyclinol (**236**) in 87% yield over just two steps. K10 clay was employed a second time in order to effect an S_N' rearrangement of compound **236**, in which methanol was expelled from the molecule and (+)-hexacyclinol was thus delivered in 99% yield. This route produced (+)-hexacyclinol in an overall yield of 30% and in 11 steps from phenol **193**. A single-crystal X-ray analysis of racemic hexacyclinol, obtained using the same protocols as described here but proceeding from (±)-**196**, was carried out and reported as part of the Porco synthesis.

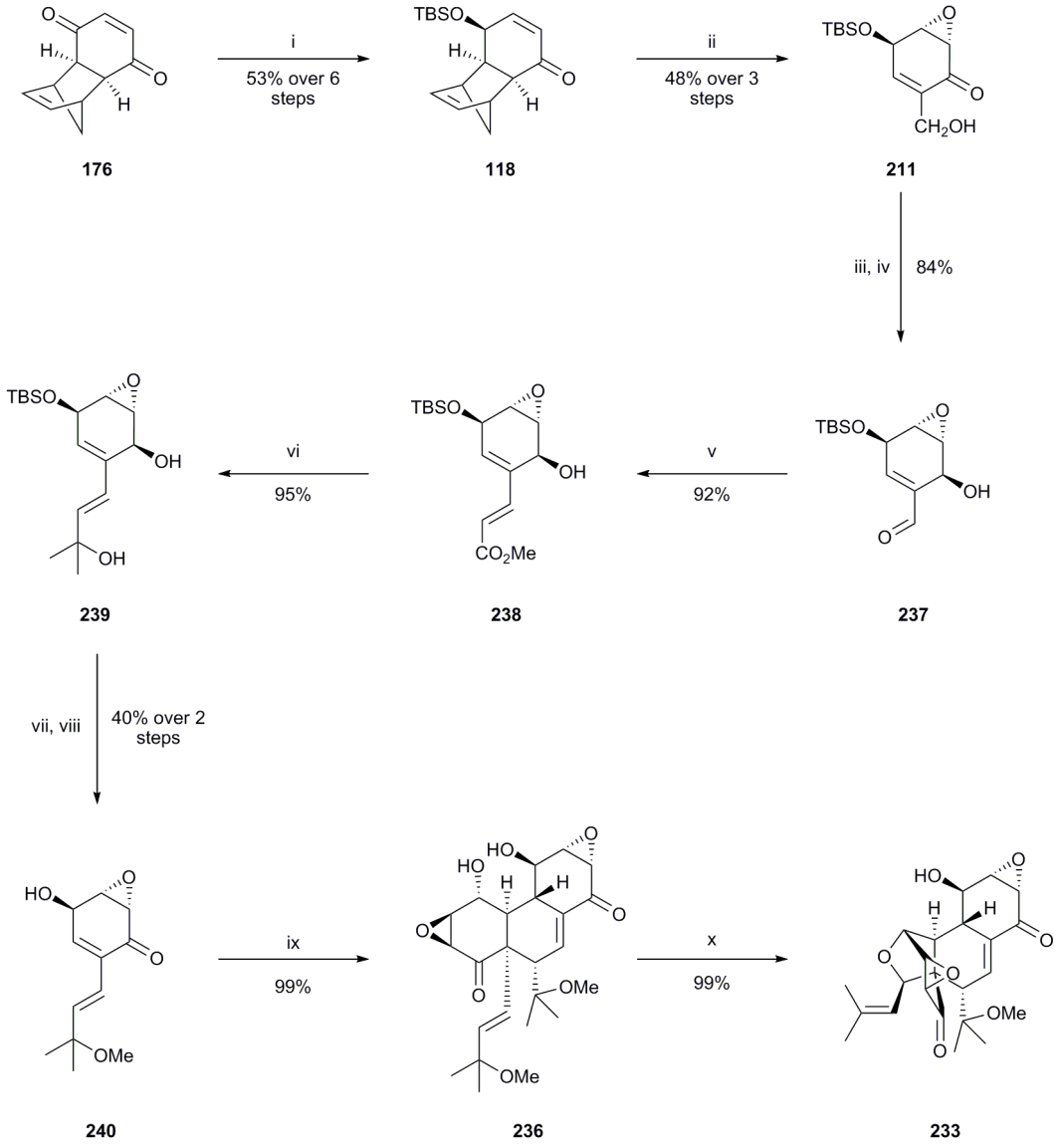


Scheme 3-15: *Reagents* (i) As described by Porco *et al*.;[95] (ii) K10 clay; (iii) TESCl, 2,6-lutidine, DMAP; (iv) $[Pd_2(dba)_3]$, $AsPh_3$, (*E*)-tributyl(3-methoxy-3-methylbut-1-enyl)stannane; (v) $Et_3N{\cdot}3HF$; (vi) neat; (vii) K10 clay.

Mehta's total synthesis of (+)-hexacyclinol (2008)

Mehta's 2008 total synthesis of (+)-hexacyclinol started with the commercially-available Diels-Alder adduct (**176**) of cyclopentadiene and *p*-benzoquinone (Scheme 3-16). Following a procedure described previously by Ogasawara and Kamikubo, γ-siloxy enone **118** was obtained from adduct **176** in six steps and in a completely enantioselective manner.[85] Advanced intermediate **211**, common to an earlier synthesis of the related natural product (+)-panepophenanthrin, was obtained from enone **118** in a further three steps and *via* protocols described in Section 3.1.2.[98] This enone was reduced in a diastereoselective manner with di*iso*butyl aluminium hydride to afford an intermediate diol. Due to the steric demands of the reagent, only the primary allylic alcohol of this intermediate was oxidized by the *N*-oxoammonium salt generated from TEMPO, CuCl and oxygen. The resulting aldehyde **237** was engaged in a Wittig reaction with a stabilized ylide and thereby afforded dienyl ester **238**, which was converted into tertiary alcohol **239** *via* exposure of the former species to excess methyl lithium in THF. Allylic oxidation of the secondary alcohol within compound **239** re-established the ketone moiety. A Dowex-1 resin-mediated S_N1 reaction involving methanol as nucleophile resulting in the tertiary hydroxyl group being replaced by a methyl ether. Deprotection of the silyl ether residue also took place under these conditions and thereby affording the desired monomeric precursor **240** to (+)-hexacyclinol. This material subsequently engaged in a highly selective Diels-Alder dimerization reaction that yielded (+)-pre-hexacyclinol (**236**) in 99% yield. Dowex-1 resin was then used to trigger a subsequent S_N' displacement and cyclization

reaction that afforded (+)-hexacyclinol (**233**) in 99% yield. This seventeen-step synthesis of (+)-hexacyclinol afforded the target compound in an overall yield of 7% from adduct **176**.



Scheme 3-16: *Reagents* (i) As described by Ogasawara and Kamikubo;[85] (ii) As described by Mehta *et al.*;[98] (ii) di*iso*butylaluminium hydride; (iii) TEMPO, CuCl, O_2; (iv) $Ph_3P{=}CHCO_2Me$; (v) MeLi; (vi) MnO_2; (vii) Dowex-1, MeOH; (viii) neat; (ix) Dowex-1.

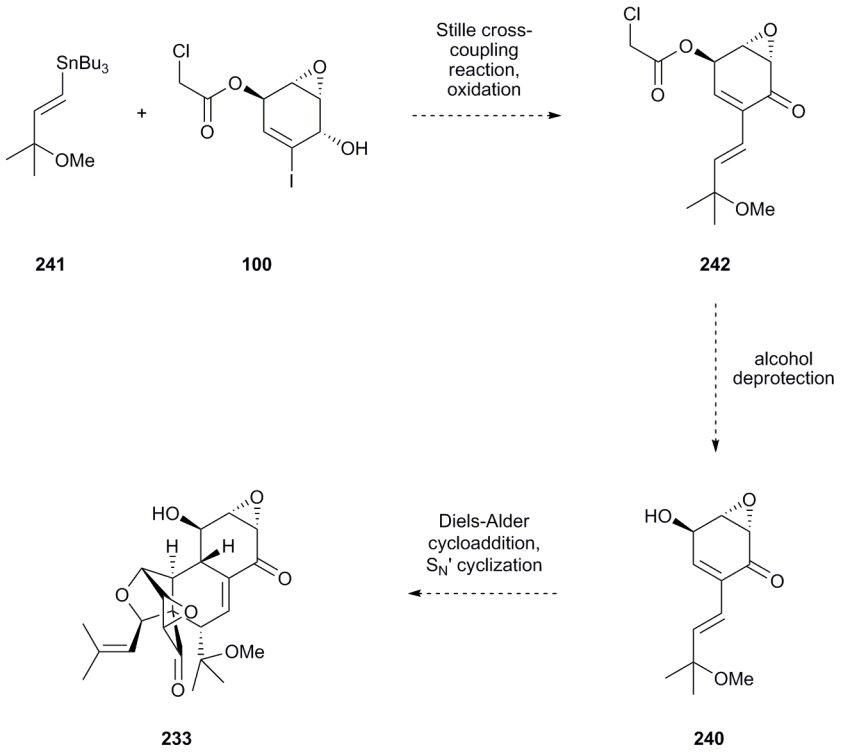
3.5 SYNTHETIC STRATEGY ASSOCIATED WITH THE PRESENT SYNTHESIS OF (+)-HEXACYCLINOL

3.5.1 Proposed synthetic route to (+)-hexacyclinol

Preceding syntheses of (+)-hexacyclinol established epoxyquinol monomer **240** as a key intermediate from which the target is obtained *via* two high-yielding and probably biomimetic reactions. As such, the synthetic efforts of the author were directed toward the rapid attainment

of compound **240** (Scheme 3-17). Porco *et al*. installed the alkenyl side-chain of alcohol **240** *via* the Stille cross-coupling reaction of alkenyl bromide **234** with stannane **241**.[93] In accord with this approach, it was proposed that alkenyl iodide **100** would serve as a suitable substrate for a cross-coupling reaction with the same stannane. As described in Section 2.3, iodide **100** was obtained from commercially available diol **90** in three steps and 40% yield.[103] It was anticipated that oxidation of the cross-coupling product would furnish diene **242** and that cleavage of the protecting group from this compound would deliver pivotal alcohol **240**. Analogous conditions to those applied in earlier syntheses would, at this juncture, be applied in order to generate (+)-hexacyclinol (**233**) from monomeric precursor **240**.

As (+)-hexacyclinol has been shown to exhibit interesting biological activity, the development of an abbreviated synthesis of this compound is of considerable importance.[94,138] The implementation of a Pd[0]-catalyzed cross-coupling reaction is especially desirable as it lends itself to the incorporation of alternate side-chains, in the event that analogues of the the hexacyclinol are sought. As well, the removal of the chloroacetyl protecting group has been shown to proceed under mild conditions and avoids the use of hazardous fluoride reagents.



Scheme 3-17: *Proposed synthetic route to (+)-hexacyclinol.*

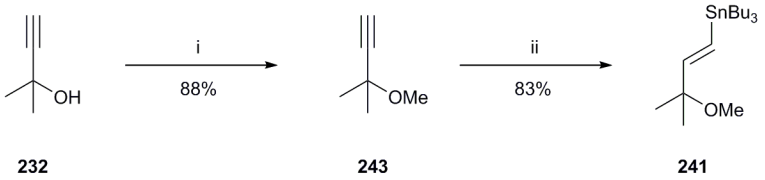
3.6 TOTAL SYNTHESIS OF (+)-HEXACYCLINOL

Though iodide **100** is available in just three steps and in enantiomerically pure form from the abundant *cis*-dihydrocatechol **90**, it was necessary to prepare stannane **241** in order to carry out

the intended cross-coupling reaction. Efforts to do so are described immediately below and followed by a discussion of the Stille cross-coupling and subsequent reactions *en route* to (+)-hexacyclinol.

3.6.1 Preparation of the relevant *trans*-alkenyl stannane

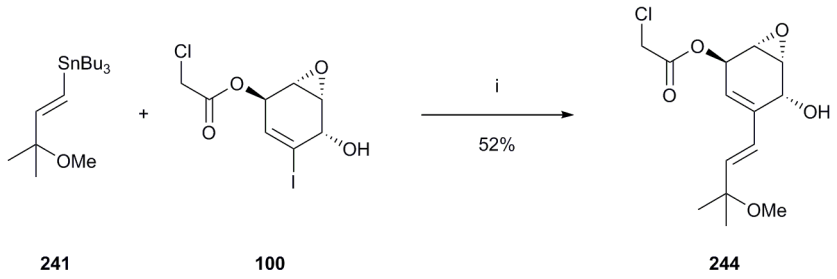
The required alkenyl stannane **241** was obtained from 2-methylbut-3-yn-2-ol *via* sequential *O*-methylation of the substrate with dimethyl sulfate and sodium hydride in DMF then the palladium[0]-catalyzed hydrostannylation of the ensuing ether **243** with tri-*n*-butyltin hydride (Scheme 3-18).[93,142] Ether **243** was obtained, after purification by distillation, as a clear, colorless oil, bp = 78-80 °C @ 760 mm Hg (lit.[142] bp = 78-80 °C @ 760 mm Hg). As the relatively high molecular weight of stannane **241** precludes its purification by distillation, a modification was made to the stoichiometry of the reaction so as minimize the production of distannyl biproducts. In particular, a 5% excess of alkyne **243** relative to tri-*n*-butyltin hydride was used. This modification reduced the prevalence of distannyl by-products to such an extent that satisfactory purification of the product was achieved by passing it through TLC-grade silica gel as a solution in *n*-hexane, followed by concentration of the filtrate. In this way, column chromatography on expensive reverse-phase silica gel was avoided.



Scheme 3-18: *Reagents and conditions* (i) $(MeO)_2SO_2$, NaH, DMF, 0→20 °C, 0.75 h; (ii) *n*-Bu_3SnH, Pd[0], THF, 20 °C, 1 h.

3.6.2 The Stille cross-coupling reaction

The pivotal cross-coupling reaction of stannane **241** and iodide **100** (Scheme 3-19) was complete after 0.3 h at 50 °C and diene **244** was isolated from the reaction mixture as a pale-orange oil in 47% yield. The ester carbonyl gives rise to a signal at δ 166.5 in the ^{13}C NMR spectrum while, due to their chemical inequivalence, the carbons of the two geminal methyl groups produced two signals at δ 25.8 and 25.3. The IR spectrum displayed an absorption band due to hydroxyl stretching at 3401 cm^{-1}. The intense absorption appearing at 1761 cm^{-1} in the same spectrum was attributed to stretching of the ester carbonyl stretching mode. The EI mass spectrum displayed a 3:1 ratio of molecular ions at *m/z* 302 and 304, as would be expected for a mono-chlorinated compound. An accurate mass measurement on the former species established that it was of the anticipated composition, *viz*. $C_{14}H_{19}{}^{35}ClO_5$.



241 100 244

Scheme 3-19: *Reagents and conditions* (iii) Pd[0], Ph_3As, CuI, THF, 50 °C, 0.3 h

The ^{1}H NMR spectrum of diene **244** (Figure 3-7) displayed two mutually coupled doublets arising from the protons on the *exo*-cyclic alkene residue. The magnitude of the coupling (J = 16.2 Hz) clearly indicates that the *E*-configured alkene has been obtained. The proton appended to the cyclic olefin is shielded by 0.8 ppm following the cross-coupling reaction, an effect that is partly attributed to greater steric compression in the coupling product.

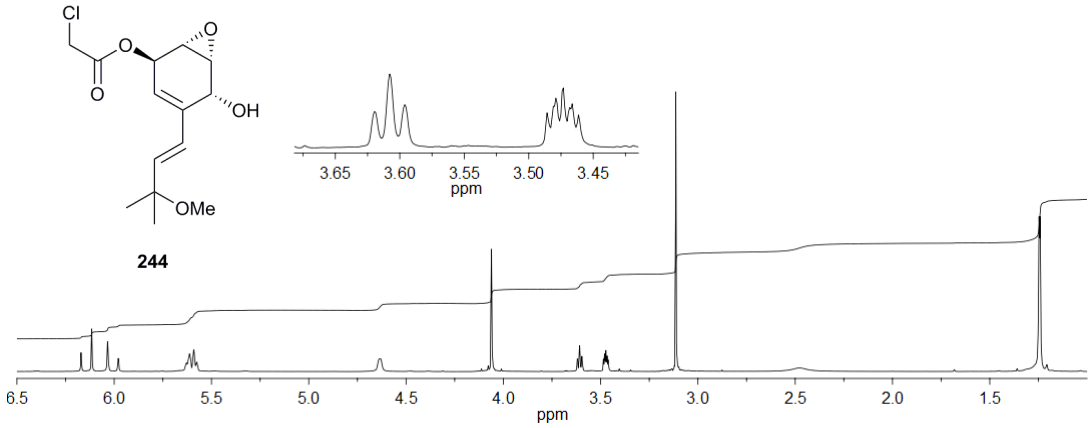
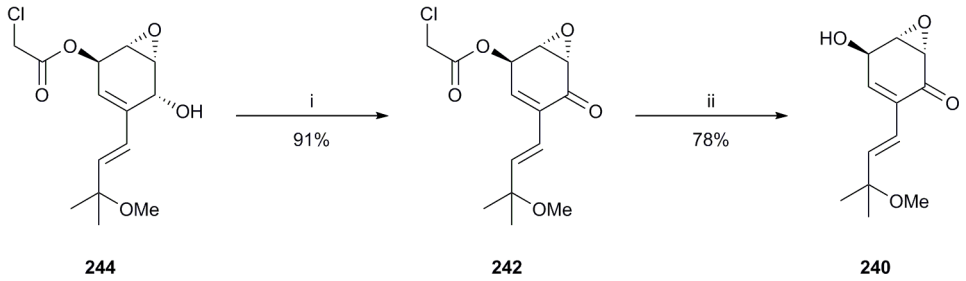


Figure 3-7: *300 MHz ^{1}H NMR spectrum of diene **244** (recorded in $CDCl_3$).*
Inset: An expansion of a region from the spectrum.

3.6.3 Alcohol oxidation and ester cleavage

Dienyl ether **244**, obtained from the cross-coupling reaction shown in Scheme 3-19, was oxidized with pyridinium dichromate and thereby affording the anticipated enone **242** as a clear, colorless oil in 91% yield. Methanolic zinc (II) acetate was used to cleave the chloroacetyl residue within compound **242** and alcohol **240** was thereby obtained in 78% yield (Scheme 3-20). The specific rotation of this material {$[\alpha]_D$ −139.9 (*c* 2.05, $CHCl_3$)} was of the same sign but of slightly lower magnitude than that described in the literature {lit.[94] $[\alpha]_D$ −170.5 (*c* 0.54, $CHCl_3$)}.



244 **242** **240**

Scheme 3-20: *Reagents and conditions* (i) PDC, AcOH, CH_2Cl_2, 20 °C, 1 h; (ii) $Zn(OAc)_2.2H_2O$, MeOH, 20 °C, 1 h.

The IR spectrum recorded on alcohol **240** displays a broad absorption band at 3385 cm^{-1} and this is assigned to the hydroxyl stretching mode of the alcohol moiety while a sharp absorption at 1687 cm^{-1} is consistent with the presence of the α,β-unsaturated ketone. In the ^1H NMR spectrum of this compound the C5 proton resonates as a doublet of doublets at δ 6.48 (J = 5.4 and 3.0 Hz). This and other spectral features were in accord with those reported by Mehta *et al.*[94] Once again, the geminally-related methyl groups gave rise to two distinct signals in the ^{13}C NMR spectrum (Figure 3-8), both appearing at δ 29.7.

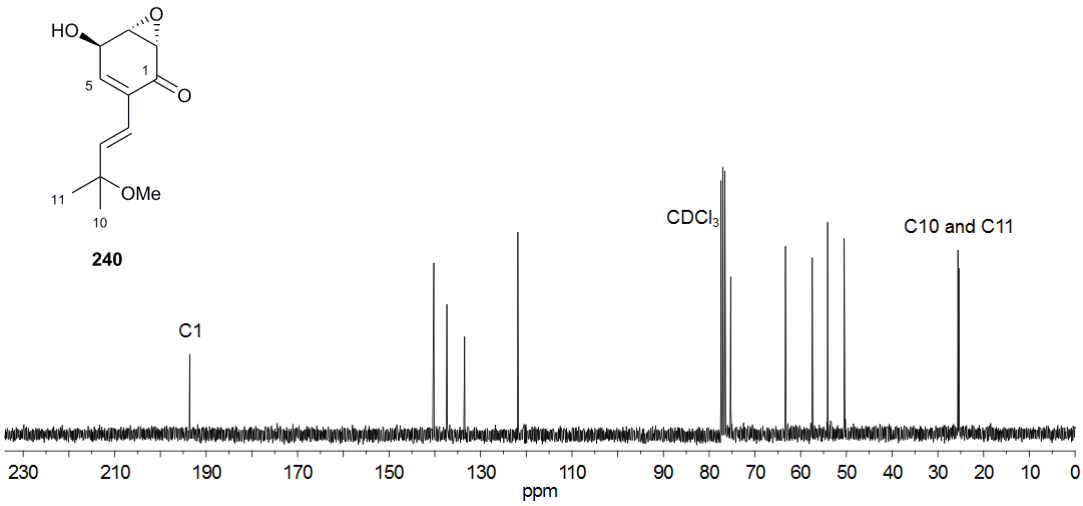
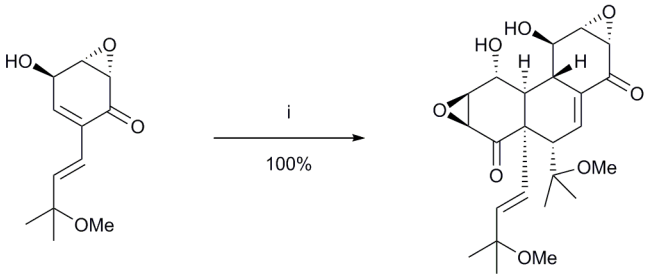


Figure 3-8: *75 MHz ^{13}C NMR spectrum of alcohol **240** (recorded in $CDCl_3$).*

3.6.4 The Diels-Alder dimerization reaction

Neat samples of alcohol **240** stored at 20 °C for 72 h underwent the anticipated *exo*-Diels-Alder dimerization reaction and delivered (+)-pre-hexacyclinol (**236**) quantitatively. This was consistent with literature descriptions of the process.[93,94] The direction of the specific rotation recorded on the product of the cycloaddition reaction {$[\alpha]_D$ +30.8 (*c* 2.35, $CHCl_3$)} was inverted relative to the monomeric precursor and agreed closely with that reported by Porco *et al.*[93] but was higher in magnitude than that reported by Mehta *et al.*[94]



240 **236**

Scheme 3-21: *Reagents and conditions* (i) neat, 20 °C, 72 h.

The ^{13}C NMR spectrum recorded on (+)-pre-hexacyclinol agreed closely with that reported by both Porco and Mehta although some small discrepancies were apparent. Comparisons of ^{13}C NMR data arising from the present work with those reported in the literature are shown in Table 3-3. In the spectrum recorded by Porco *et al*., 22 of the 24 chemically unique carbon signals were observed with two "missing" signals presumably obscured or overlapping with others. Mehta *et al.* observed all 24 carbon signals while in the current work 23 signals were observed. The particular signal absent in the present study was also obscured in the spectrum recorded by Porco *et al*. Nevertheless, the remaining chemical shift values extracted from the ^{13}C NMR spectrum were in good agreement with the literature values; $\Delta\delta$ values are $\leq$ 0.8 ppm for 20 of the 23 observed signals and the average $\Delta\delta$ value, for all of the observed signals, was less than 0.5 ppm (Table 3-3).

Table 3-3: *Comparison of the ^{13}C NMR data recorded for different synthetically-derived samples of (+)-pre-hexacyclinol (**236**)*

Acquired by Porco *et al*.[93] (δ_C) (75 MHz, $CDCl_3$)	Acquired by Mehta *et al.*[94] (δ_C) (75 MHz, $CDCl_3$)	Acquired by Pinkerton *et al.*[104] (δ_C) (75 MHz, $CDCl_3$)
203.0	202.6	203.4
194.7	194.4	194.9
138.6	138.5	138.5
134.2	138.4	137.9
130.9	133.9	134.5
not observed	130.7	130.8
77.5	77.3	77.0
75.5	75.2	75.5
69.6	69.7	68.4
68.1	68.0	67.5
61.6	61.5	61.3
59.3	59.0	59.4
55.5	55.2	55.5
53.7	53.5	53.6
53.6	53.5	53.5
not observed	52.9	not observed
50.6	50.4	50.6
49.9	49.5	50.3
48.7	48.5	48.4
46.5	46.3	46.3
26.3	26.1	26.0
25.3	25.1	25.1
24.7	24.6	24.4
23.6	23.5	23.1

The ^{13}C NMR spectrum recorded on (+)-pre-hexacyclinol is depicted in Figure 3-9. The signals due to the carbonyl carbons C6 and C1 appear at δ 203.4 and 194.9, respectively. In addition, the olefinic carbons give rise to a set of four signals between δ 140 and 130. Signal broadening is apparent for the remaining signals, all of which appear below δ 78 and this effect is probably due to the slowed rotation of the methyl ether sidechains about the core due to their severely sterically-crowded environments.

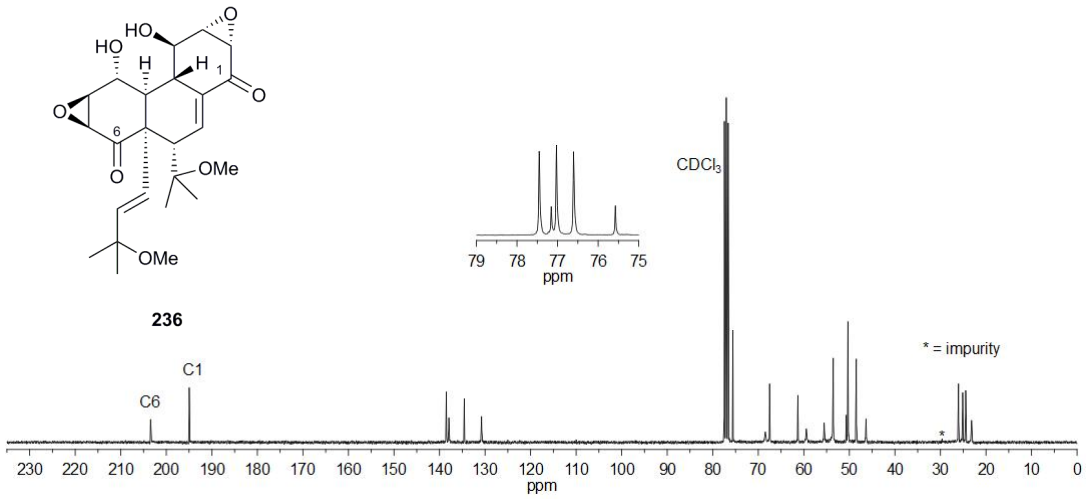


Figure 3-9: *75 MHz ^{13}C NMR spectrum of (+)-pre-hexacyclinol (recorded in $CDCl_3$).*
Inset: An expansion of a region from the spectrum.

The ^{1}H NMR spectrum recorded on (+)-pre-hexacyclinol is in good agreement with the analogous spectra recorded by Porco and Mehta.[93,94] A comparison of these sets of spectral data is presented in Table 3-4. In each data set, signals integrating to 31 protons are observed. In the current work, signals ascribed to the two hydroxyl protons appear at relatively low-field positions. The relevant chemical shifts of these signals are δ 4.07 and 3.72, as opposed to δ 3.05 and 2.82 reported by Porco *et al*. and δ 3.02 and 2.76 ppm reported by Mehta *et al*.

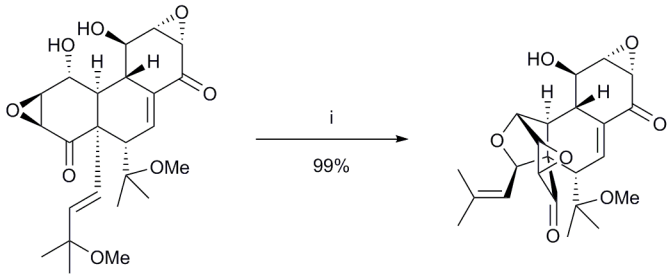
The IR spectrum of (+)-pre-hexacyclinol exhibits a broad absorption band at 3424 cm^{-1} that is attributed to hydroxyl groups while absorptions due to the two epoxy ketones overlap at 1699 cm^{-1}. The ESI mass spectrum displays an $[M+Na]^{+}$ ion at *m/z* 471 and accurate mass measurement of this species established that it was of the expected composition, *viz*. $C_{24}H_{32}NaO_8$.

Table 3-4: *Comparison of the ^{1}H NMR data recorded for different synthetically-derived samples of (+)-pre-hexacyclinol (**236**)*

Acquired by Porco *et al*.[93] (δ_H) (300 MHz, $CDCl_3$)	Acquired by Mehta *et al*.[94] (δ_H) (300 MHz, $CDCl_3$)	Acquired by Pinkerton *et al*.[104] (δ_H) (300 MHz, $CDCl_3$)
6.71 (dd, *J* = 4.3 and 2.6 Hz, 1H)	6.73 (dd, *J* = 4.3 and 2.6 Hz, 1H)	6.69 (m, 1H)
5.80 (d, *J* = 16.9 Hz, 1H)	5.80 (d, *J* = 17.0 Hz, 1H)	5.84 (d, *J* = 16.8 Hz, 1H)
5.41 (d, *J* = 16.9 Hz, 1H)	5.44 (d, *J* = 17.0 Hz, 1H)	5.40(d, *J* = 16.8 Hz, 1H)
4.43 (broad s, 1H)	4.46 (broad s, 1H)	4.44 (broad s, 1H)
4.15 (dd, *J* = 8.2 and 4.7 Hz, 1H)	4.17 (d, *J* = 8.4 Hz, 1H)	4.21 (m, 1H)
		4.07 (d, *J* = 5.4 Hz, 1H)
		3.72 (d, *J* = 5.4 Hz, 1H)
3.57 (d, *J* = 3.6 Hz, 1H)	3.59 (d, *J* = 3.3 Hz, 1H)	3.60 (m, 2H)
3.55 (d, *J* = 3.4 Hz, 1H)	3.57 (d, *J* = 3.4 Hz, 1H)	
3.45 (m, 2H)	3.46 (m, 2H)	3.49 (d, *J* = 3.6 Hz, 1H)
		3.44 (broad s, 1H)
3.32 (d, *J* = 3.4 Hz, 1H)	3.34 (d, *J* = 3.2 Hz, 1H)	3.35 (d, *J* = 3.6 Hz, 1H)
3.18 (s, 3H)	3.20 (s, 3H)	3.16 (s, 3H)
3.05 (m, 4H)	3.07 (s, 3H)	3.05 (s, 3H)
	3.02 (broad s, 1H)	not observed
2.82 (d, *J* = 6.4 Hz, 1H)	2.76 (broad s, 1H)	not observed
2.68 (ddd, *J* = 10.8, 5.2 and 3.4 Hz, 1H)	2.72-2.68 (m, 1H)	2.67 (m, 1H)
2.58 (t, *J* = 4.6 Hz, 1H)	2.61 (t, *J* = 4.7 Hz, 1H)	2.52 (broad s, 1H)
1.23 (s, 3H)	1.25 (s, 3H)	1.22 (s, 6H)
1.22 (s, 3H)	1.25 (s, 3H)	
1.20 (s, 3H)	1.23 (s, 3H)	1.19 (s, 3H)
1.12 (s, 3H)	1.14 (s, 3H)	1.12 (s, 3H)

3.6.5 The acid-catalyzed intramolecular S_N' reaction – Conversion of (+)-pre-hexacyclinol into (+)-hexacyclinol

Exposure of (+)-pre-hexacyclinol (**236**) to a catalytic amount of acid, in this case that present in untreated chloroform or deuterochloroform, induced the previously described S_N' reaction to form (+)-hexacyclinol (Scheme 3-22). The reaction proceeded almost quantitatively at 20 °C over 72 h to afford the desired product, (+)-hexacyclinol, as a white, crystalline solid, mp 176-178 °C (lit.[94] mp 176-178 °C) following removal of the reaction solvent under vacuum.



236 **233**

Scheme 3-22: *Reagents and conditions* (vii) H^+/D^+, $CDCl_3$, 20 °C, 72 h.

X-ray crystallographic analysis of (+)-hexacyclinol afforded the ORTEP shown in Figure 3-10. This structure matches that of the natural product and is in accord with a previous X-ray crystal structure obtained on the racemic material and published by Porco *et al*.[93]

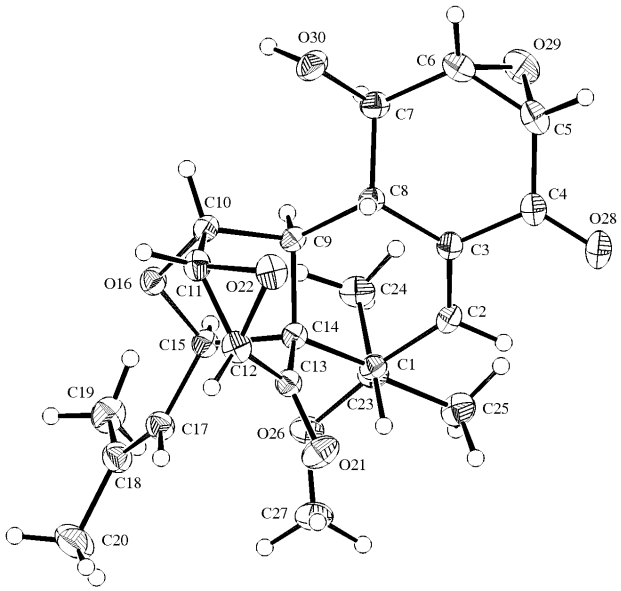


Figure 3-10: *ORTEP derived from the single-crystal X-ray analysis of (+)-hexacyclinol.*

The specific rotation measured on (+)-hexacyclinol {$[\alpha]_D$ +129.6 (*c* 0.36, methanol)} was in the same direction and of very similar magnitude to that described by Mehta *et al.* {$[\alpha]_D$ +130.9 (*c* 0.42, methanol)}.[94] The ^{13}C and ^{1}H NMR spectra (Figure 3-11 and Figure 3-12, respectively) were in accord with those described for the natural product.[93,94,138] The two signals due to the carbonyl carbons C6 and C1 appear at δ 203.1 and 193.2, respectively, in the ^{13}C NMR spectrum. As the latter carbonyl moiety is conjugated with an alkene, the associated carbonyl carbon (C1) remains relatively more shielded.

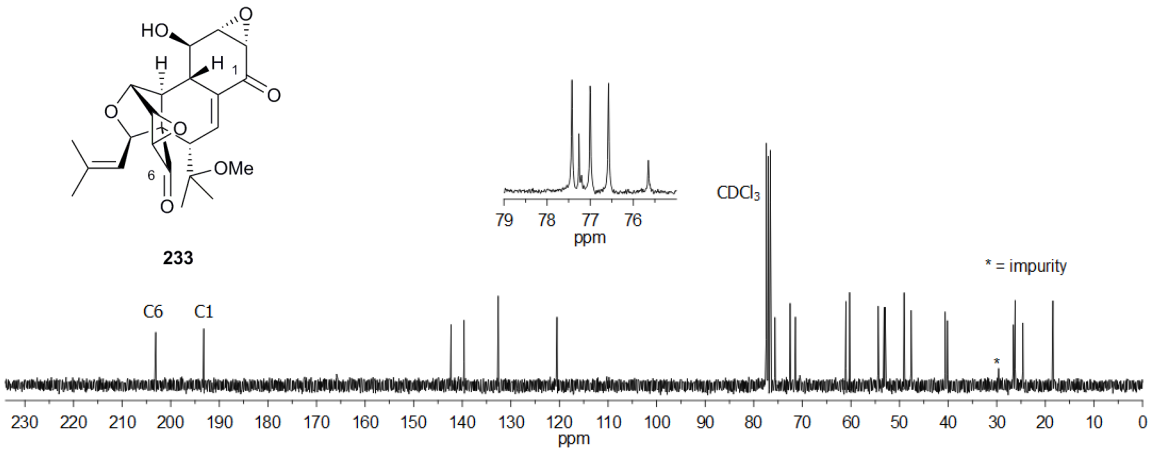


Figure 3-11: *75 MHz ^{13}C NMR spectrum of (+)-hexacyclinol (recorded in $CDCl_3$).*
Insets: An expansion of a region of the spectrum.

The ^{1}H NMR spectrum recorded on (+)-hexacyclinol (Figure 3-12) was entirely consistent with that obtained from the natural product and from other synthetically-derived samples.[93,94,138] The olefinic proton at the β-position of the enone appeared as a doublet of doublets at δ 6.72 (J = 6.0 and 2.4 Hz). A lone doublet (J = 9.3) at δ 5.47 is assigned to the proton at C17 while the resonance at δ 3.02 was attributed to the methyl ether.

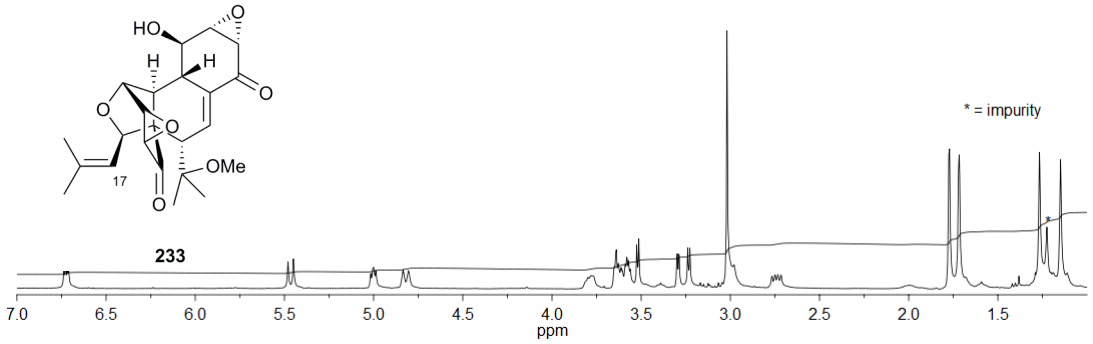


Figure 3-12: *300 MHz ^{1}H NMR spectrum of (+)-hexacyclinol (recorded in $CDCl_3$).*

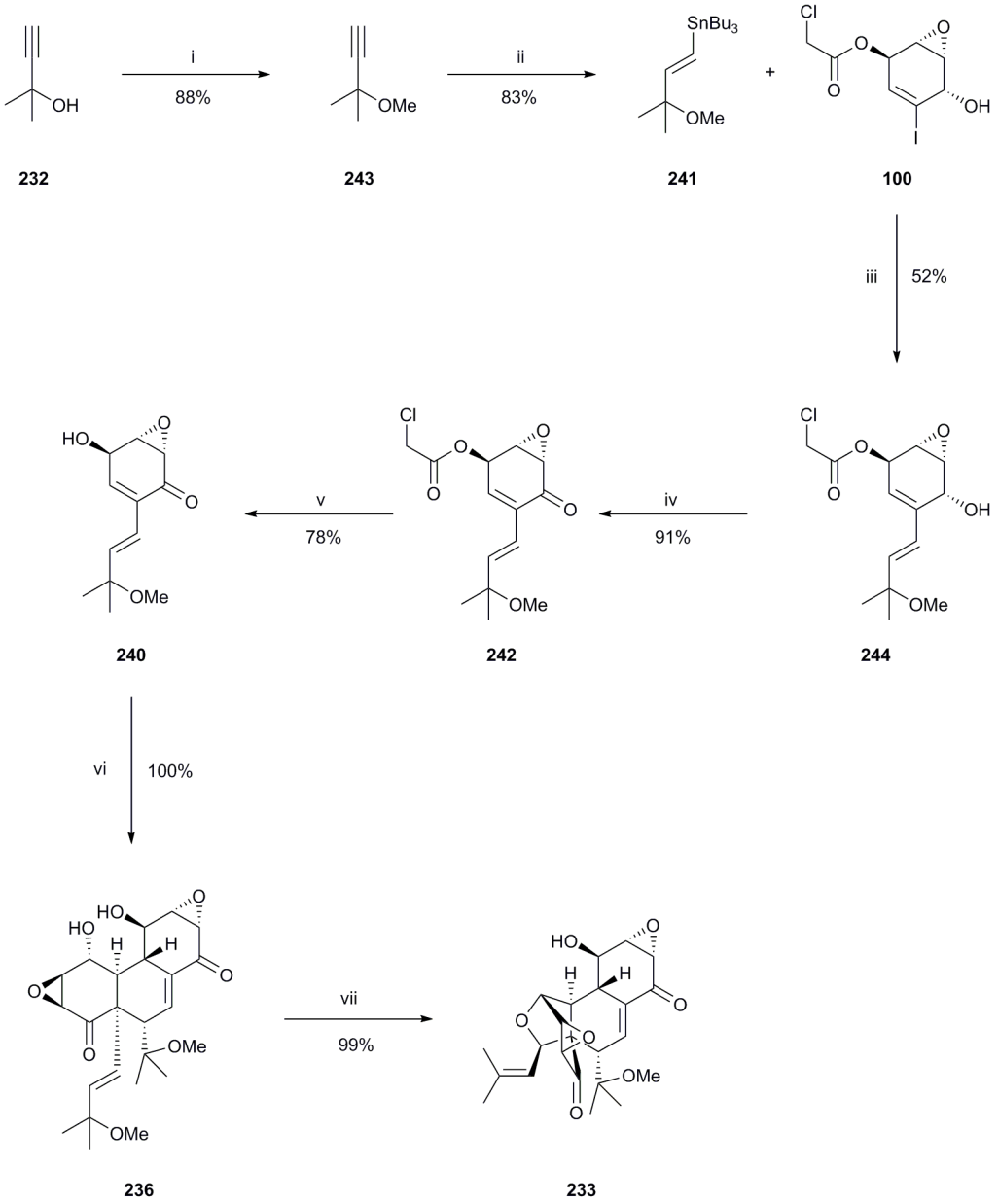
A comparison of the ^{13}C and ^{1}H NMR data recorded on naturally- and synthetically-derived (+)-hexacyclinol (**233**) is presented in Table 3-5. The two ^{13}C NMR spectra are in close agreement with the Δδ values for relevant ^{13}C nuclei being ≤ 0.6 ppm and an average value of 0.2 ppm. The chemical shifts of those ^{1}H NMR signals that are not part of the complex multiplet appearing between δ 3.70 and 3.54 show Δδ values ≤ 0.1 ppm.

Table 3-5: *Comparison of the ^{13}C and ^{1}H NMR data recorded on naturally- and synthetically-derived (+)-hexacyclinol (**233**)*

^{13}C NMR Data Natural **233** (δ_C) (75 MHz, $CDCl_3$)[138]	^{13}C NMR Data Synthetic **233** (δ_C) (75 MHz, $CDCl_3$)	^{1}H NMR Data Natural **233** (δ_H) (300 MHz, $CDCl_3$)[138]	^{1}H NMR Data Synthetic **233** (δ_H) (300 MHz, $CDCl_3$)
202.9	203.1	6.73 (dd, *J* = 5.3 and 2.4 Hz, 1H)	6.72 (dd, *J* = 6.0 and 2.4 Hz, 1H)
192.8	193.2	5.46 (d, *J* = 10.1 Hz, 1H)	5.47 (d, *J* = 9.3 Hz, 1H)
142.2	142.3	4.99 (broad d, *J* = 5.2 Hz, 1H)	5.00 (m, 1H)
139.0	139.6	4.82 (d, *J* = 10.1 Hz, 1H)	4.82 (d, *J* = 9.3 Hz, 1H)
132.5	132.6	3.80 (broad dd, *J* = 9.5 and 1.5 Hz, 1H)	
120.7	120.5	3.64 (m, 1H)	3.70-3.54 (complex m, 3H)
77.3	77.3	3.59 (d, *J* = 5.3 Hz, 1H)	
75.8	75.7	3.55 (m, 1H)	
72.7	72.5	3.51 (dd, *J* = 2.9 and 0.5 Hz, 1H)	3.52 (d, *J* = 3.6 Hz, 1H)
71.5	71.4	3.29 (d, *J* = 3.2 Hz, 1H)	3.29 (d, *J* = 2.7 Hz, 1H)
61.0	61.0	3.23 (broad d, *J* = 3.5 Hz, 1H)	3.24 (d, *J* = 3.6 Hz, 1H)
60.5	60.3	3.02 (s, 3H)	3.02 (s, 3H)
54.5	54.4	2.74 (dd, *J* = 7.8 and 5.2 Hz, 1H)	2.74 (dd, *J* = 9.9 and 5.1 Hz, 1H)
53.2	53.2	1.77 (s, 3H)	1.77 (s, 3H)
53.1	53.0	1.72 (s, 3H)	1.72 (s, 3H)
49.1	49.1	1.26 (s, 3H)	1.27 (s, 3H)
47.8	47.6	1.15 (s, 3H)	1.15 (s, 3H)
40.9	40.6		
40.4	40.1		
26.6	26.6		
26.1	26.2		
24.7	24.6		
18.6	18.4		

The hydroxyl groups of compound **233** produce a broad absorption band at 3433 cm^{-1} in the IR spectrum while carbonyl stretches produce a pair of partially overlapping absorption bands at 1711 and 1698 cm^{-1}. The cyclic alkene gives rise to an absorption band at 1626 cm^{-1}, the intensity of which is enhanced by conjugation with the adjacent ketone. The ESI mass spectrum displays an $[M+Na]^+$ ion at *m/z* 439 and accurate mass measurement of this species established that it was of the expected composition, *viz*. $C_{23}H_{28}NaO_7$.

Scheme 3-23 provides a summary of the reaction sequence employed in the synthesis of (+)-hexacyclinol from the epoxyquinol synthon **100**. By such means, the target (+)-hexacyclinol was obtained in an overall yield of 15% and in eight steps from the commercially available *cis*-dihydrocatechol **90**.



Scheme 3-23: *Reagents and conditions* (i) $(MeO)_2SO_2$, NaH, DMF, 0-20 °C, 0.75 h; (ii) *n*-Bu_3SnH, Pd[0], THF, 20 °C, 1 h; (iii) Pd[0], Ph_3As, CuI, THF, 50 °C, 0.3 h; (iv) PDC, AcOH, CH_2Cl_2, 20 °C, 1 h; (v) $Zn(OAc)_2.2H_2O$, MeOH, 20 °C, 1 h; (vi) neat, 20 °C, 72 h; (vii) H^+/D^+, $CDCl_3$, 20 °C, 72 h.

The present work represents the shortest total synthesis of (+)-hexacyclinol reported to date (Table 3-6). Although the overall yield for this sequence is surpassed by the earlier work of Porco *et al.*, the average yield per reaction step in the newer sequence is, at 79%, substantial. Conveniently, every step in the synthesis of (+)-hexacyclinol described here is conducted at standard temperatures and pressures, with the exception of the Stille cross-coupling reaction for which only mild heating is required.

Table 3-6: *A comparison of the key features associated with the three reported syntheses of (+)-hexacylinol*

Lead author	Publication date	Longest linear sequence	Overall yield (%)
Porco[93]	2006	eleven steps	30
Mehta[94]	2008	seventeen steps	7
Present work[104]	2009	eight steps	15

3.7 CONCLUSIONS AND FUTURE WORK

Commercially available iodo-diol **100**, obtained *via* the chemoenzymatic dihydroxylation of iodobenzene, served as the starting material for rapid total syntheses of the dimeric epoxyquinol-derived natural products, (+)-panepophenanthrin and (+)-hexacyclinol. Seven and eight steps afforded the target compounds in 11 and 15% yields, respectively. In each case, the work here represents the shortest synthetic route to these structurally complex natural products. As well, more than half of the carbon atoms that comprise the framework of these target molecules are drawn from abundant iodobenzene. Given the benign nature of the reaction conditions employed in these sequences and their brevity, the methodology described here lends itself to the further investigation of these and related compounds for medicinal applications.

CHAPTER FOUR

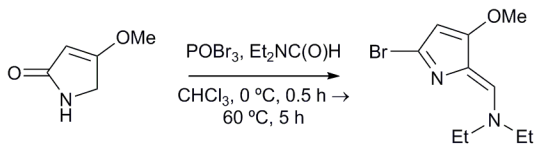
EXPERIMENTAL PROCEDURES ASSOCIATED WITH WORK DESCRIBED IN CHAPTERS ONE, TWO AND THREE

4.1 GENERAL PROCEDURES

Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Varian Gemini machine operating at 300 MHz. Unless otherwise specified, spectra were acquired at 20 °C in deuterochloroform ($CDCl_3$) that had been stored over anhydrous sodium carbonate. Chemical shifts are recorded as δ values in parts per million (ppm). Infrared spectrums (ν_{max}) were normally recorded on a Perkin–Elmer 1800 Series FTIR Spectrometer and samples were analyzed as thin films on KBr plates (for liquids) or as a KBr disc (for solids). Low-resolution ESI mass spectra were recorded on a Micromass–Waters LC-ZMD single quadrupole liquid chromatograph-mass spectrometer while low- and high-resolution EI mass spectra were recorded on a VG Fisons AUTOSPEC three-sector double-focusing instrument. Melting points were measured on Reichert hot-stage microscope or a Stanford Research Systems Optimelt – Automated Melting Point System and are uncorrected. Analytical thin layer chromatography (TLC) was performed on aluminum-backed 0.2 mm thick silica gel 60 F254 plates as supplied by Merck. Eluted plates were visualized using a 254 nm UV lamp and/or by treatment with a suitable dip followed by heating. These dips included a mixture of vanillin: sulfuric acid: ethanol (1 g : 1 g : 18 mL) or phosphomolybdic acid : ceric sulfate : sulfuric acid (conc.) : water (37.5 g : 7.5 g : 37.5 g : 720 mL). The retardation factor (Rf) values cited here have been rounded at the first decimal point. Flash chromatographic separations were carried out following protocols defined by Still *et al.*[143] with silica gel 60 (0.040–0.0063 mm) as the stationary phase and using the AR- or HPLC-grade solvents indicated. Starting materials and reagents were generally available from the Sigma–Aldrich, Merck, TCI, Strem or Lancaster Chemical Companies and were either used as supplied or, in the case of liquids, distilled when required. Drying agents and other inorganic salts were purchased from the AJAX, BDH or Unilab Chemical Companies. THF, dichloromethane, acetonitrile and benzene were dried using a Glass Contour solvent purification system that is based upon a technology originally described by Grubbs *et al.*[144] Spectroscopic grade solvents were used for all analyses. Where necessary, reactions were performed under a nitrogen or argon atmosphere.

4.2 EXPERIMENTAL PROCEDURES FOR CHAPTER ONE

2-bromo-6-diethylamino-4-methoxy-1-azafulvene (40)



21 **40**

A solution phosphorus oxybromide (12.4 g, 43.3 mmol) in chloroform (15 mL) was added to solution of diethyl formamide (5.8 mL, 52.1 mmol) in chloroform (5 mL) at 0 ºC. The resulting suspension was stirred at 0 ºC for 30 minutes, and the solvent was removed by rotary evaporation to obtain the Vilsmeier complex as a white solid. After drying in vacio for 20 min, chloroform (10 mL) was added to the solid and cooled to 0 ºC. A solution of **21** (2.0 g, 17.7 mmol) in chloroform (20 mL) was added dropswise and the mixture was heated at 60 ºC for 5 h. The mixture was poured onto ice (75 mL), and the pH of the aqueous solution was adjusted to pH 7-8 by treatment with a 2M aqueous solution of potassium carbonate. Ethyl acetate (40 mL) was added and the mixture was filtered over Celite® to remove the black solid containing the phosphorus salts. The layers were separated and the aqueous later was extracted with ethyl acetate (3 × 100 mL). The combined organic fractions were washed with brine (3 × 200 mL) before being dried ($MgSO_4$), filtered and concentrated under reduced pressure. Subjection of the ensuing material to flash chromatography (silica, 1:4 v/v ethyl acetate/*n*-hexane elution) and concentration of the relevant fractions (Rf 0.3 in 20% v/v ethyl acetate/*n*-hexane) afforded azafulvene **40** (2.3 g, 49%) as an oil that solidified on standing at 18 ºC as a tan solid, mp 38-40 ºC (lit. mp 38-40 ºC) (lit.[25] mp = 38-40 ºC).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.04 (s, 1H), 5.63 (s, 1H), 4.11 (q, *J* = 7.5 Hz, 2H), 3.78 (s, 3H), 3.42 (q, *J* = 7.5 Hz, 2H), 1.33-1.23 (complex m, 6H).

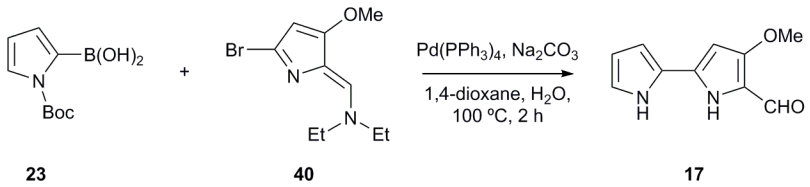
^{13}C NMR (75 MHz, $CDCl_3$) δ 165.1, 138.5, 133.3, 120.2, 96.2, 57.9, 51.0, 44.4, 14.5, 12.4.

IR (KBr) ν_{max} 3430, 2950, 1635, 1530, 1410, 1280, 1115, 910, 720 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/z* 260 and 258 ($M^{+\bullet}$, both 95%), 231 and 229 (97 and 100), 215 and 217 (95 and 90).

HREIMS Found: $M^{+\bullet}$, 258.0364. $C_{10}H_{15}{}^{79}BrN_2O$ requires $M^{+\bullet}$, 258.0368.

4-methoxy-2,2'-bipyrrole-5-carboxaldehyde (17)



Triphenylphosphine (460 mg, 1.7 mmol) was added to a magnetically stirred suspension of palladium(II) acetate (86 mg, 0.38 mmol) in degassed toluene (1.5 mL) and the mixture was heated to 70 ºC for 20 min under an atmosphere of nitrogen. *N*-Boc-pyrrole-2-boronic acid (**23**) (1.22 g, 5.8 mmol) and azafulvene **40** (1.0 g, 3.9 mmol) were dissolved in 1:9 v/v water/1,4-dioxane (15 mL). This solution was degassed, purged with nitrogen gas, and then transferred to the suspension of $Pd(PPh_3)_4$ in toluene. Sodium carbonate (1.2 g, 11.3 mmol) was added and the resulting mixture was stirred at 100 ºC for 2 h and then poured onto water (100 mL). The pH of the mixture was lowered to pH 7 with HCl (2M aqueous solution). The brown precipitate was recovered by filtration and washed with water (10 mL), cold acetone (10 mL) and cold diethyl ether (2 × 10 mL) to reveal the title aldehyde **17** (700 mg, 95%) as a yellow solid, mp 222-225 ºC (lit.[25] mp 224-227 ºC).

^{1}H NMR (300 MHz, d_6-DMSO) δ 11.50 (s, 1H), 11.20 (s, 1H), 9.30 (s, 1H), 6.92 (s, 1H), 6.76 (s, 1H), 6.28 (s, 1H), 6.13 (t, *J* = 3.0 Hz, 1H), 3.85 (s, 3H).

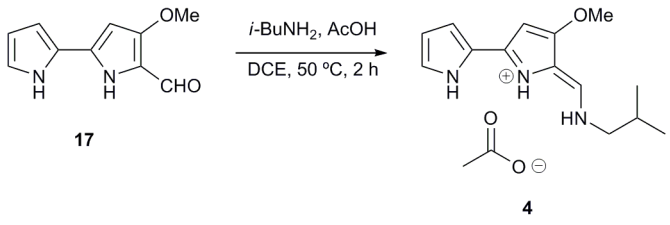
^{13}C NMR (75 MHz, d_6-DMSO) δ 171.7, 158.8, 133.3, 123.74, 120.4, 117.4, 109.4, 108.4, 90.9, 57.8.

IR (KBr) ν_{max} 3248, 3200, 1606, 1362, 1162, 1014, 772 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/z* 190 ($M^{+\bullet}$, 100%), 161 (40), 143 (40), 108 (50), 92 (60), 91 (65).

HREIMS Found: $(M+H)^+$, 191.0821. $C_{10}H_{10}N_2O_2$ requires $(M+H)^+$, 191.0821.

Tambjamine C (4)

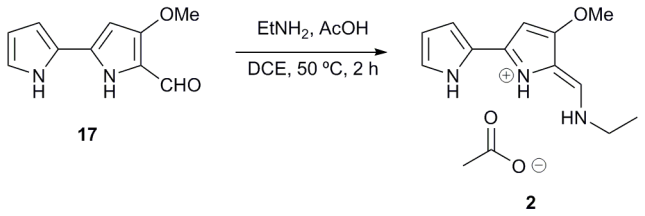


Acetic acid (150 µL, 2.6 mmol) and isobutyl amine (260 µL, 2.6 mmol) were added to a magnetically stirred solution of aldehyde **17** (50 mg, 0.26 mmol) in DCE (5 mL). The ensuing mixture was stirred at 18 °C for 3 h, diluted with dichloromethane (5 mL), rinsed with water (3

× 10 mL) then concentrated under reduced pressure to afford the title alkaloid **4**[6] (77 mg, 97%) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 11.1 (broad s, 2H), 7.28 (s, 1H), 7.04 (m, 1H), 6.70 (m, 1H), 6.24 (m, 1H), 5.95 (s, 1H), 3.90 (s, 3H), 3.24 (d, *J* = 6.6 Hz, 1H), 2.08 (s, 3H, $CH_3CO_2^-$), 1.98 (m, 1H), 0.99 (d, *J* = 6.6 Hz, 6H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 178.8, 163.5, 142.8, 140.8, 123.6, 122.9, 112.5, 110.7, 109.9, 91.0, 58.5, 58.0, 29.4, 24.4, 19.6.
IR (KBr) ν_{max} 3440, 3190, 2960, 1670, 1620, 1525 cm^{-1}.
Mass spectrum (EI, 70 eV) *m/z* 245 ($M^{+\bullet}$, 90%), 230 (40), 202 (100).
HREIMS Found: $M^{+\bullet}$, 245.1527. $C_{14}H_{19}N_3O$ requires $M^{+\bullet}$, 245.1528.

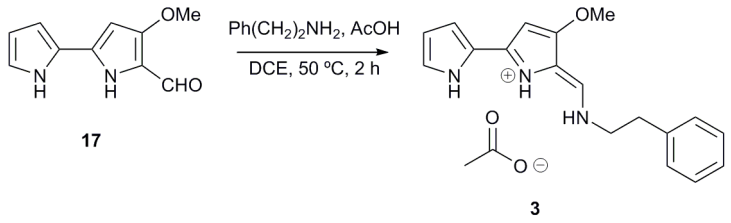
Tambjamine E (2)



Acetic acid (150 µL, 2.6 mmol) and ethylamine (1.3 mL of a 2 M solution in methanol, 2.6 mmol) were added to a magnetically stirred solution of aldehyde **17** (50 mg, 0.26 mmol) in DCE (5 mL). The ensuing mixture was stirred at 50 °C for 3 h, diluted with dichloromethane (5 mL), rinsed with water (3 × 10 mL) then concentrated under reduced pressure to afford the title alkaloid **2**[145] (50 mg, 69%) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 11.1 s, 2H), 7.30 (s, 1H), 6.83 (s, 1H), 6.57 (m, 1H), 6.19 (m, 1H), 5.94 (s, 1H), 3.85 (s, 3H), 3.32 (q, *J* = 7.5 Hz, 2H), 2.10 (s, 3H, $CH_3CO_2^-$), $CH_3CO_2^-$), 1.18 (t, *J* = 7.5 Hz, 3H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 179.2, 162.9, 143.6, 140.5, 124.9, 121.8, 114.2, 110.4, 109.6, 91.6, 68.0, 45.6, 24.7, 15.7.
IR (KBr) ν_{max} 3198, 2926, 1669, 1616, 1528, 1343, 1261, 1162, 1136, 1117, 1035, 1012, 965, 771, 733 cm^{-1}.
Mass spectrum (EI, 70 eV) *m/z* 217 ($M^{+\bullet}$, 100%), 202 (75), 175 (70).
HREIMS Found: $M^{+\bullet}$, 217.1205. $C_{12}H_{15}N_3O$ requires $M^{+\bullet}$, 217.1215.

Tambjamine F (3)



Acetic acid (150 µL, 2.6 mmol) and β-phenethylamine (330 µL, 2.6 mmol) were added to a magnetically stirred solution of aldehyde **17** (50 mg, 0.26 mmol) in DCE (5 mL). The ensuing mixture was stirred at 50 °C for 3 h, diluted with dichloromethane (5 mL), rinsed with water (3 × 10 mL) then concentrated under reduced pressure to afford the title alkaloid **3**[145] (91 mg, 98%) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 12.2 (s, 2H), 7.32-7.19 (complex m, 5H), 7.08 (s, 1H), 6.68 (m, 1H), 6.25 (m, 1H), 5.89 (s, 1H), 3.81 (s, 3H), 3.64 (t, *J* = 7.2 Hz, 2H) 3.00 (t, *J* = 7.2 Hz, 2H), 2.10 (s, 3H, $CH_3CO_2^-$).

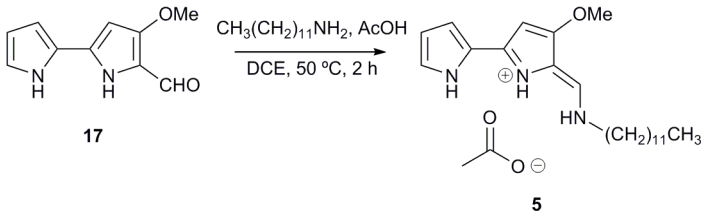
^{13}C NMR (75 MHz, $CDCl_3$) δ 179.1, 163.5, 142.8, 140.5, 137.4, 128.8, 128.6, 126.6, 123.5, 122.9, 112.5, 110.8, 110.0, 91.1, 58.0, 52.3, 36.6, 24.6.

IR (KBr) ν_{max} 3220, 2926, 1680, 1625, 1534, 1407, 1312, 1188, 1119, 1036, 1011, 884, 775, 745, 707, 643 cm^{-1}.

Mass spectrum (ESI, 70 eV) *m/z* 217 [$(M+H)^+$, 100%].

HRESIMS Found: $(M+H)^+$, 294.1597. $C_{18}H_{19}N_3O$ requires $(M+H)^+$, 294.1606.

BE-18591 (5)



Acetic acid (150 µL, 2.6 mmol) and dodecylamine (480 mg, 2.6 mmol) were added to a magnetically stirred solution of aldehyde **17** (50 mg, 0.26 mmol) in DCE (5 mL). The ensuing mixture was stirred at 50 °C for 3 h, diluted with dichloromethane (5 mL), rinsed with water (3 × 10 mL) then concentrated under reduced pressure to afford the title alkaloid **5**[1] (110 mg, 100%) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 11.4 (s, 2H), 7.26 (s, 1H), 7.00 (m, 1H), 6.64 (m, 1H), 6.21 (m, 1H), 5.89 (s, 1H), 3.83 (s, 3H), 3.37 (t, *J* = 7.1 Hz, 2H), 2.06 (s, 3H, $CH_3CO_2^-$), 1.66 (m, 2H), 1.22 (m, 18H), 0.85 (t, *J* = 6.8 Hz, 3H).

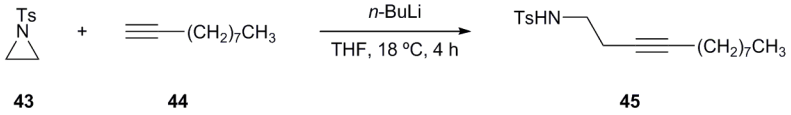
^{13}C NMR (75 MHz, $CDCl_3$) δ 179.0, 163.2, 142.6, 140.5, 123.4, 123.1, 112.3, 110.9, 109.9, 91.0, 58.0, 50.9, 31.8, 30.3, 29.5, 29.4, 29.3, 29.2, 29.1, 26.4, 24.6, 22.6, 14.0.

IR (KBr) ν_{max} 3436, 2923, 1665, 1604, 1550, 1510, 1465, 1309, 1250, 1164, 1137, 1044, 1009, 967, 808, 729 cm^{-1}.

Mass spectrum (ESI, 70 eV) *m/z* 358 [$(M+H)^+$, 100%], 358 (50), 360 (10), 60 (10).

HRESIMS Found: $(M+H)^+$, 358.2853. $C_{22}H_{35}N_3O$ requires $(M+H)^+$, 358.2858.

***N*-tosyldodec-3-yn-1-amine (45)**



n-Butyllithium (56 mL of a 1.6 M solution in hexanes, 89.6 mmol) was added, dropwise, to a magnetically stirred solution of 1-decyne (**44**) (16.0 mL, 88.9 mmol) in anhydrous THF (50 mL) at 18 ºC under a nitrogen atmosphere. The resulting solution was stirred at 18 ºC for 20 minutes before being treated with a solution of aziridine **43** (4.32 g, 21.9 mmol) in THF (25 mL). The ensuing mixture was stirred at 18 ºC for a further 4 h then treated with sodium bicarbonate (50 mL of a saturated aqueous solution) and extracted with diethyl ether (3 × 100 mL). The organic extract was dried ($MgSO_4$), filtered and concentrated under reduced pressure. Subjection of the ensuing residue to flash chromatography (silica, 55% v/v ethyl acetate/n-hexane elution and concentration of the appropriate fractions (R_f 0.37, 11:9 v/v ethyl acetate/*n*-hexane) afforded the *title alkyne **45*** (3.1 g, 42 %) as a yellow oil that solidified on standing at 18 ºC as a clear crystalline solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, *J* = 8.4 Hz, 2H), 7.31 (d, *J* = 8.4 Hz, 2H), 4.70 (t, *J* = 6.3 Hz, 1H), 3.04 (dt, *J* = 6.3 and 6.3 Hz, 2H), 2.43 (s, 3H), 2.29 (tt, *J* = 6.3 Hz and 2.4 Hz, 2H), 2.09 (tt, *J* = 7.1 and 2.4 Hz, 2H), 1.50-1.20 (m, 12H), 0.88 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 143.4, 136.9, 129.7, 127.0, 83.2, 75.6, 42.1, 31.8, 29.1, 29.1, 28.9, 28.8, 22.6, 21.5, 19.8, 18.6, 14.1.

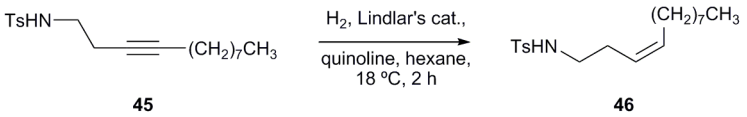
IR (KBr) ν_{max} 3268, 2926, 2853, 1319, 1165, 1155, 1076, 815, 551 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/z* 693 [$(2M+Na)^+$, 15%], 374 [$(M+K)^+$, 10), 358 [$(M+Na)^+$, 100], 336 [$(M+H)^+$, 80].

HRESIMS Found: $(M-H)^-$, 334.1832. $C_{19}H_{28}NO_2S$ requires $(M-H)^-$, 334.1841.

Microanalysis Calculated for $C_{19}H_{29}NO_2S$ C, 68.02; H, 8.71; N, 4.17. Found C, 68.24; H, 8.47; N, 3.90%.

(*Z*)-*N*-tosyldodec-3-en-1-amine (46)

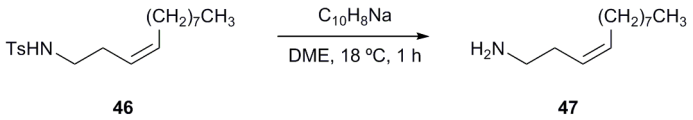


45 **46**

Lindlar's catalyst (34 mg) was added to a magnetically stirred solution of alkyne **45** (2.21 g, 6.58 mmol) and quinoline (0.35 mL, 2.5 mmol) in *n*-hexane (10 mL) and the ensuing mixture was stirred at 18 ºC under an atmosphere of hydrogen for 2 h then filtered and concentrated under under reduced pressure to provide a yellow oil. This oil was subsequently redissolved in diethyl ether (30 mL) and rinsed with hydrochloric acid (3 × 30 mL of a 0.01 M aqueous solution) and brine (30 mL) then dried ($MgSO_4$), filtered and concentrated under reduced pressure to yield the *title olefin **46*** (2.2 g, 99%) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, *J* = 8.4 Hz, 2H), 7.30 (d, *J* = 8.4 Hz, 2H), 5.49 (m, 1H), 5.15 (m, 1H), 4.40 (t, *J* = 6.2 Hz, 1H), 2.96 (m, 2H), 2.42 (s, 3H), 2.19 (m, 2H), 1.94 (m, 2H), 1.25 (m, 12H), 0.88 (t, J = 6.6 Hz, 3H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 143.3, 134.0, 129.7, 127.1, 124.4, 42.7, 31.8, 29.5, 29.5, 29.3, 27.3, 22.7, 21.5, 14.1 (two signals obscured or overlapping).
IR (KBr) ν_{max} 3283, 2925, 2854, 1328, 1161, 1095, 814, 663, 551 cm^{-1}.
Mass spectrum (EI, 70 eV) *m/z* 697 [$(2M+Na)^+$, 10%], 360 [$(M+Na)^+$, 80], 338 [$(M+H)^+$, 100].
HRESIMS Found: $(M+H)^+$, 338.2165. $C_{19}H_{31}NO_2S$ requires $(M+H)^+$, 338.2154.

(*Z*)-dodec-3-en-1-amine (47)



46 **47**

Sodium (274 mg, 11.9 mmol) was added to a magnetically stirred solution of naphthalene (904 mg, 7.1 mmol) in 1,2-dimethoxyethane (15 mL). The resulting mixture was stirred at 18 ºC for 150 min. After this time, a solution of alkene **46** (598 mg, 1.77 mmol) was added and the ensuing mixture was stirred at 18 ºC for 1 h and then quenched by the addition of ethanol (1 mL). The solvent was removed under reduced pressure and the residue was subjected to chromatography (silica, methanol elution). Concentration of the appropriate fractions (R_f 0.15, methanol) afforded the *title amine **47*** (200 mg, 72%) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.49 (m, 1H), 5.32 (m, 1H), 2.72 (t, *J* = 6.9 Hz, 2H), 2.19 (m, 2H), 2.02 (m, 2H), 1.96 (s, 2H), 1.25 (m, 12H), 0.87 (t, *J* = 6.8 Hz, 3H).
^{13}C NMR (75 MHz, CDCl3) δ 132.4, 126.4, 31.8, 31.9, 31.2, 29.7, 29.5, 29.3 (×2), 27.4, 22.7, 14.1.

IR (KBr) ν_{max} 3431, 2924, 2854, 1577, 1467, 1379, 1326, 722 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/z* 184 [$(M+H)^+$, 65%], 111 (40), 97 (95), 83 (100), 69 (100), 54 (95).

HREIMS Found: $(M+H)^+$, 184.2066. $C_{12}H_{25}N$ requires $(M+H)^+$, 184.2065.

Compound 6

Acetic acid (60 µL, 1.1 mmol) and amine **47** (195 mg, 1.1 mmol) were added to a magnetically stirred solution of aldehyde **17** (50 mg, 0.26 mmol) in DCE (10 mL) maintained under nitrogen. The ensuing mixture was stirred at 18 °C for 2 h then diluted with water (10 mL) and dichloromethane (10 mL). The separated organic layer was washed with water (2 × 10 mL) then concentrated under reduced pressure to afford a yellow oil. Subjection of this material to flash chromatography (silica, ethyl acetate → methanol gradient elution) and concentration of the appropriate fractions (R_f = 0.15 in ethyl acetate) afforded the title alkaloid **6**[2] (35 mg, 32%) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 11.4 (s, 2H), 7.28 (s, 1H), 7.04 (m, 1H), 6.69 (m, 1H), 6.25 (m, 1H), 5.94 (s, 1H), 5.54 (m, 1H), 5.34 (m, 1H), 3.89 (s, 3H), 3.45 (t, *J* = 6.9 Hz, 2H), 2.45 (m, 2H), 2.09 (s, 3H, $CH_3CO_2^-$), 2.02 (m, 2H), 1.22 (m, 12H), 0.85 (t, *J* = 6.8 Hz, 3H).

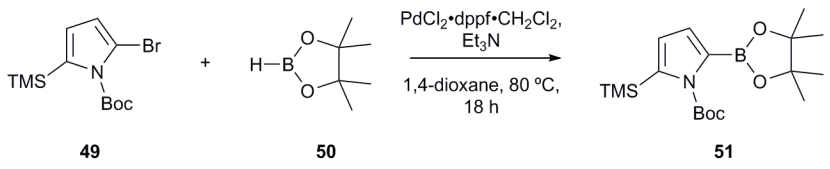
^{13}C NMR (75 MHz, $CDCl_3$) δ 179.0, 163.5, 142.9, 140.6, 134.1, 123.9, 123.7, 123.1, 112.5, 110.9, 110.1, 91.2, 58.2, 50.8, 31.8, 29.7, 29.5, 29.3, 29.3, 28.4, 27.4, 24.5, 22.6, 14.1.

IR (KBr) ν_{max} 3431, 2925, 2853, 1666, 1601, 1576, 1528, 1426 cm^{-1}.

Mass spectrum (ESI, 70 eV) *m/z* 356 [$(M+H)^+$, 100%].

HRESIMS Found: $(M+H)^+$, 356.2694. $C_{22}H_{33}N_3O$ requires $(M+H)^+$, 356.2702.

***tert*-Butyl 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-(trimethylsilyl)-pyrrole-1-carboxylate (51)**



[1,1′-Bis(diphenylphosphino)ferrocene]dichloropalladium(II)/CH_2Cl_2 complex (71 mg, 0.087 mmol) was suspended in a magnetically stirred solution of pyrrole **49** (1.0 g, 3.15 mmol)

and triethylamine (1.4 mL, 10 mmol) in 1,4-dioxane (15 mL). Pinacolborane (**50**) (0.7 mL, 4.8 mmol) was added and the ensuing suspension was stirred for at 80 ºC for 20 h. After this time, the mixture was diluted with *n*-hexane and rinsed with water (3 × 50 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford the *title pyrrole **51*** (0.91 g, 80%) as tan crystals, mp = 86 ºC.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.72 (d, *J* = 3.0 Hz, 1H), 6.44 (d, *J* = 3.0 Hz, 1H), 1.62 (s, 9H), 1.32 (s, 12H), 0.26 (s, 9H).

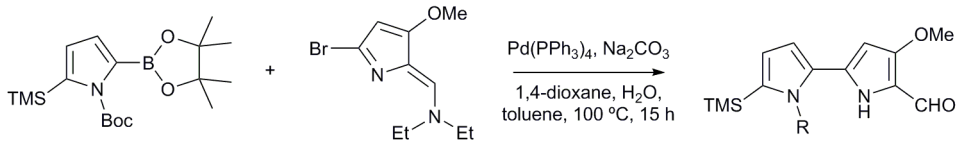
^{13}C NMR (75 MHz, $CDCl_3$) δ 151.5, 140.4, 124.0, 123.1, 84.2, 83.4, 27.9, 24.8, -0.1 (one signal obscured or overalapping).

IR (KBr) ν_{max} 2980, 1735, 1534, 1460, 1340, 1206, 1145, 1081, 841, 688 cm-1.

Mass spectrum (EI, 70 eV) *m/z* 365 ($M^{+\bullet}$, 25%), 294 (80), 265 (78), 250 (99), 194 (100), 83 (50), 57 (80).

HREIMS Found: $M^{+\bullet}$, 365.2193. $C_{18}H_{32}BNO_4Si$ requires $M^{+\bullet}$, 365.2194.

3-Methoxy-5-(1-(*tert*-butoxycarbonyl)-5-(trimethylsilyl)-pyrrol-2-yl)pyrrole-2-carbaldehyde (52) and 3-Methoxy -5-(5-(trimethylsilyl)-pyrrol-2-yl)pyrrole-2-carbaldehyde (53)



51 **40** **52** R=Boc **53** R=H

$Pd(PPh_3)_4$ was generated *in situ* by adding triphenyl phosphine (1.0 g, 3.8 mmol) to a magnetically stirred suspension of palladium(II) acetate (191 mg, 0.85 mmol) in degassed toluene (3 mL) then heating the ensuing mixture to 70 °C for 0.33 h under nitrogen. In a separate flask, a solution of pyrrole **51** (4.2 g, 11.5 mmol) and azafulvene **40** (2.0 g, 7.7 mmol) in water/1,4-dioxane (30 mL of a 1:9 v/v mixture) was degassed, purged with nitrogen and then transferred to the suspension of $Pd(PPh_3)_4$ in toluene prepared as described above. Sodium carbonate (2.4 g, 22.6 mmol) was then added to the reaction mixture that was then stirred at 100 °C for 15 h before being cooled and poured into water (100 mL). The mixture was neutralized throught the addition of HCl (2M aqueous solution) then extracted with dichloromethane (3 × 100 mL). the combined organic extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure and the ensuing brown oil was subjected to flash chromatography (silica, 1:3 v/v ethyl acetate/hexane elution). In this manner two fractions, A and B, were obtained.

Concentration of fraction A (R_f = 0.4 in 1:3 ethyl acetate/hexane) afforded *aldehyde* ***52*** (660 mg, 23%) as white crystals, mp = 147 °C.

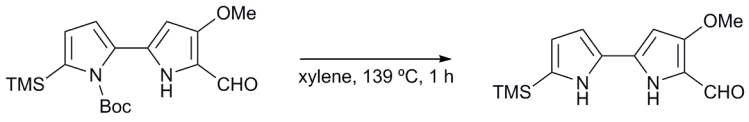
^{1}H NMR (300 MHz, $CDCl_3$) δ 9.50 (s, 1H), 8.90 (s, 1H), 6.47 (d, *J* = 3.3 Hz, 1H), 6.43 (d, *J* = 3.3 Hz, 1H), 5.91 (d, *J* = 2.7 Hz, 1H), 3.88 (s, 3H), 1.41 (s, 9H), 0.30 (s, 9H).
IR (KBr) ν_{max} 3215, 1748, 1608, 1363, 1321, 1167, 1137, 1020, 844 cm^{-1}.
Mass spectrum (EI, 70 eV) *m/z* 362 ($M^{+\bullet}$, 15%), 306 (70), 291 (45), 263 (65), 262 (100), 247 (80), 57 (74).
HREIMS Found: $M^{+\bullet}$, 362.1661. $C_{18}H_{26}N_2O_4Si$ requires $M^{+\bullet}$, 362.1662.

This somewhat unstable compound was used immediately in the next step of the reaction sequence.

Concentration of fraction B (R_f = 0.3 in 1:3 ethyl acetate/hexane) afforded the *title aldehyde* ***53*** (1.22 g, 60%) as pale-yellow crystals, mp = 115 °C.

^{1}H NMR (300 MHz, $CDCl_3$) δ 12.01 (s, 1H), 11.90 (s, 1H), 9.18 (s, 1H), 6.70 (m, 1H), 6.46 (m, 1H), 6.03 (d, *J* = 2.7 Hz, 1H), 3.94 (s, 3H), 0.34 (s, 9H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 162.2, 137.0, 135.7, 127.0, 118.5, 118.1, 111.5, 91.4, 57.9, 0.9 cm^{-1}.
IR (KBr) ν_{max} 3259, 3191, 2955, 1595, 1344, 1247, 1166, 1020, 951, 839 cm^{-1}.
Mass spectrum (EI, 70 eV) *m/z* 262 ($M^{+\bullet}$, 100%), 247 (75).
HREIMS Found: $M^{+\bullet}$, 262.1138. $C_{13}H_{18}N_2O_2Si$ requires $M^{+\bullet}$, 262.1138.

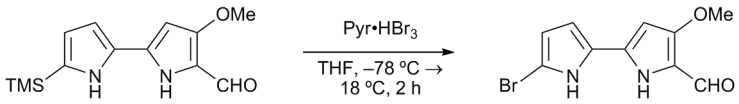
3-Methoxy-5-(5-(trimethylsilyl)-pyrrol-2-yl)pyrrole-2-carbaldehyde (53)



52 **53**

A solution of aldehyde **52** (370 mg, 1.01 mmol) in *m*-xylene (20 mL) was heated at reflux for 1 h and then cooled and concentrated under reduced pressure to give the *title aldehyde* ***53*** (270 mg, 100%) as pale-yellow crystals. This material was identical, in all respects, with an authentic sample generate as described immediately above.

3-Methoxy-5-(5-bromopyrrol-2-yl)pyrrole-2-carbaldehyde (54)



53 **54**

Pyridinium hydrobromide perbromide (730 mg, 2.3 mmol) was added, in one portion, to a magnetically stirred solution of aldehyde **53** (580 mg, 2.2 mmol) in THF (30 mL) maintained at − 78 ºC. The ensuing mixture was slowly warmed to 18 ºC, stirred at this temperature for 2 h then poured into water (100 mL) and extracted into ethyl acetate (3 × 100 mL). The combined organic extracts were diluted with ethanol (100 mL), then dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford the *title aldehyde **54*** (455 mg, 76%) as green crystals, mp = 163 ºC (dec.).

^{1}H NMR (300 MHz, d_6-DMSO) δ 11.87 (s, 1H), 11.43 (s, 1H), 9.32 (s, 1H), 6.77 (s, 1H), 6.33 (s, 1H), 6.18 (s, 1H), 3.83 (s, 3H).

^{13}C NMR (75 MHz, d_6-DMSO) δ 172.2, 158.5, 131.9, 125.3, 117.6, 111.5, 109.6, 100.3, 91.3, 57.9.

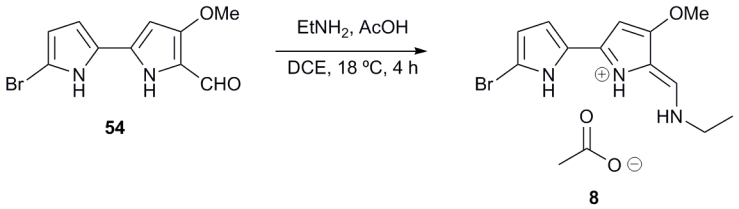
IR (KBr) ν_{max} 3245, 3209, 2923, 1594, 1519, 1504, 1427, 1342, 1298, 1166, 1021, 832, 773 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/z* 270 and 268 ($M^{+\bullet}$, both 100%), 190 (25).

HREIMS Found: $M^{+\bullet}$, 267.9847. $C_{10}H_9{}^{79}BrN_2O_2Si$ requires $M^{+\bullet}$, 267.9847.

Microanalysis Calculated for $C_{10}H_9BrN_2O_2Si$ C, 44.63; H, 3.37; N, 10.41. Found C, 44.48; H, 3.23; N, 10.15%.

Tambjamine G (8)



54 **8**

Acetic acid (110 μL, 1.9 mmol) and ethylamine (1 mL of a 2 M solution in methanol, 2.0 mmol) were added to a magnetically stirred solution of aldehyde **54** (58 mg, 0.22 mmol) in 1,2-dichloroethane (10 mL) under an atmosphere of nitrogen and the ensuing mixture was stirred at 60 ºC for 3 h then diluted with water (10 mL) and dichloromethane (10 mL). The layers were separated and the organic layer was rinsed with water (2 × 10 mL) then concentrated under reduced pressure to afford the title imine **8**[42] (69 mg, 90%) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 11.10 (broad s, 2H), 7.34 (s, 1H), 6.58 (d, *J* = 3.9 Hz, 1H), 6.16 (d, *J* = 3.9 Hz, 1H), 5.89 (s, 1H), 3.87 (s, 3H), 3.50 (t, *J* = 7.2 Hz, 2H), 2.11 (s, 3H, $CH_3CO_2^-$), 1.36 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 179.7, 163.6, 141.6, 140.9, 124.6, 113.8, 112.5, 111.2, 105.4, 91.1, 58.5, 45.9, 24.9, 15.8 cm^{-1}.

IR (KBr) ν_{max} 3429, 2930, 1668, 1610, 1526, 1407, 1344, 1260, 1157, 1011, 979, 800 cm^{-1}.

Mass spectrum (ESI, 70 eV) *m/z* 298 and 296 [$(M+H)^+$, both 100%], 217 (65), 175 (25), 140 (10), 112 (5).

HRESIMS Found: $(M+H)^+$, 296.0393. $C_{12}H_{14}{}^{79}BrN_3O$ requires $(M+H)^+$, 296.0393.

Tambjamine H (9)

9

Acetic acid (125 µL, 2.2 mmol) and *n*-propylamine (180 µL, 2.2 mmol) were added to a magnetically stirred solution of aldehyde **54** (58 mg, 0.22 mmol) in 1,2-dichloroethane (10 mL) under an atmosphere of nitrogen and the ensuing mixture was stirred at 18 ºC for 4 h then diluted with water (10 mL) and dichloromethane (10 mL). The layers were separated and the organic layer was rinsed with water (2 × 10 mL) then concentrated under reduced pressure to afford the title alkaloid **9**[42] (78 mg, 99%) as a yellow oil.

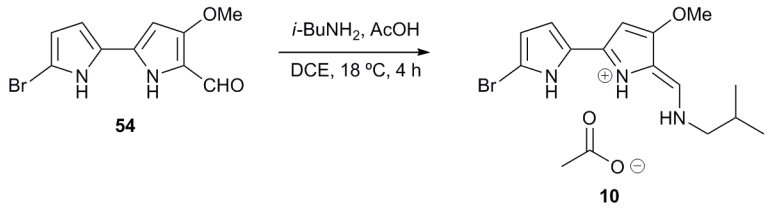
^{1}H NMR (300 MHz, $CDCl_3$) δ 11.30 (broad s, 2H), 7.29 (s, 1H), 6.53 (d, *J* = 3.6 Hz, 1H), 6.14 (d, *J* = 3.6 Hz, 1H), 5.84 (s, 1H), 3.84 (s, 3H), 3.37 (t, *J* = 7.1 Hz, 2H), 2.08 (s, 3H, $CH_3CO_2^-$), 1.71 (m, 2H), 0.96 (t, *J* = 7.4 Hz, 3H).

IR (KBr) ν_{max} 29303436, 2930, 1669, 1619, 1527, 1405, 1365, 1306, 1232, 1160, 1013, 777, 647 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/z* 311 and 309 [$(M+H)^+$, both 80%], 296 and 294 (both 30), 282 and 280 (both 50), 255 and 253 (both 30), 231 (50), 43 (100).

HREIMS Found: $(M+H)^+$, 310.0556. $C_{13}H_{16}{}^{79}BrN_3O$ requires $(M+H)^+$, 310.0555.

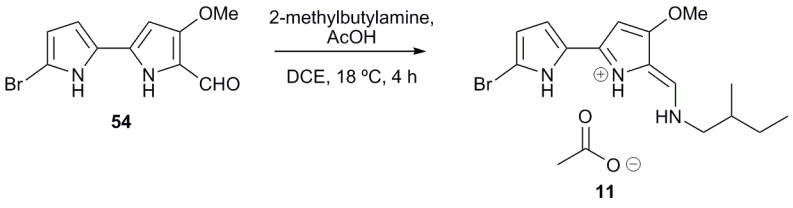
Tambjamine I (10)



Acetic acid (110 μL, 1.9 mmol) and *i*-butylamine (190 μL, 1.9 mmol) were added to a magnetically stirred solution of aldehyde **54** (50 mg, 0.19 mmol) in 1,2-dichloroethane (10 mL) under an atmosphere of nitrogen and the ensuing mixture was stirred at 18 ºC for 4 h then diluted with water (10 mL) and dichloromethane (10 mL). The layers were separated and the organic layer was rinsed with water (2 × 10 mL) then concentrated under reduced pressure to afford the title imine **10**[42] (58 mg, 81%) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 11.10 (broad s, 2H), 7.26 (s, 1H), 6.55 (d, *J* = 3.6 Hz, 1H), 6.15 (d, *J* = 3.6 Hz, 1H), 5.86 (s, 1H), 3.86 (s, 3H), 3.23 (d, *J* = 6.6 Hz, 1H), 2.08 (s, 3H, $CH_3CO_2^-$), 1.97 (m, 1H), 0.97 (d, *J* = 6.9 Hz, 6H).
^{13}C NMR (75 MHz, CDCl3) δ 179.2, 163.4, 141.5, 141.3, 124.4, 113.5, 112.1, 110.8, 105.2, 90.7, 58.6, 58.2, 29.4, 24.5, 19.7 cm^{-1}.
IR (KBr) ν_{max} 3436, 2961, 1667, 1619, 1527, 1402, 1304, 1238, 1148, 1014, 779, 646 cm^{-1}.
Mass spectrum (EI, 70 eV) *m/z* 325 and 323 ($M^{+\bullet}$, both 25%), 310 and 308 (both 10), 282 and 280 (both 30), 245 (10), 202 (15), 186 (15), 85 (62), 83 (100), 43 (53).
HREIMS Found: $M^{+\bullet}$, 324.0724. $C_{14}H_{18}{}^{79}BrN_3O$ requires $M^{+\bullet}$, 324.0711.

Tambjamine J (11)



Acetic acid (110 μL, 1.9 mmol) and 2-methylbutylamine (225 μL, 1.9 mmol) were added to a magnetically stirred solution of aldehyde **54** (52 mg, 0.19 mmol) in 1,2-dichloroethane (10 mL) under an atmosphere of nitrogen and the ensuing mixture was stirred at 18 ºC for 4 h then diluted with water (10 mL) and dichloromethane (10 mL). The layers were separated and the organic layer was rinsed with water (2 × 10 mL) then concentrated under reduced pressure to afford the title imine **11**[42] (69 mg, 90%) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 11.10 (broad s, 2H), 7.26 (s, 1H), 6.56 (d, *J* = 3.9 Hz, 1H), 6.15 (d, *J* = 3.9 Hz, 1H), 5.87 (s, 1H), 3.86 (s, 3H), 3.28 (m, 1H), 2.09 (s, 3H, $CH_3CO_2^-$), 1.73 (m, 1H), 1.46 (m, 1H), 1.19 (m, 1H), 0.93 (m, 6H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 179.3, 163.4, 141.5, 141.3, 124.4, 113.5, 112.1, 110.9, 105.3, 90.8, 58.2, 57.0, 35.8, 26.5, 24.6, 16.7, 11.1 cm^{-1}.

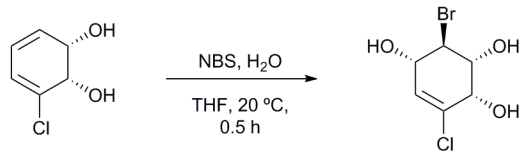
IR (KBr) ν_{max} 3436, 2963, 1663, 1613, 1525, 1402, 1337, 1228, 1157, 1008, 775, 647 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/z* 339 and 337 ($M^{+\bullet}$, both 15%), 310 and 308 (both 10), 282 and 280 (both 20), 259 (5), 202 (15), 186 (10), 45 (58), 43 (100).

HREIMS Found: $M^{+\bullet}$, 337.0789. $C_{15}H_{20}{}^{79}BrN_3O$ requires $M^{+\bullet}$, 337.0790.

4.3 EXPERIMENTAL PROCEDURES FOR CHAPTER TWO

(1*S*,2*R*,3*R*,4*S*)-3-bromo-6-chlorocyclohex-5-ene-1,2,4-triol (91)



88 **91**

A magnetically stirred solution of compound **88** (10.2 g, 69.8 mmol) in THF/H2O (4:1, v/v, 200 mL) was treated with *N*-bromosuccinimide (12.4 g, 69.8 mmol). The ensuing mixture was stirred at 20 °C for 0.5 h. $Na_2S_2O_3$ (0.48 g, 1.9 mmol) was added and after a further 0.5 h, the mixture was diluted with brine (200 mL) and extracted with diethyl ether (3 × 150 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to an amber-colored oil. Subjection of this material to flash chromatography (silica, 1:1 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.3) then gave the *title compound **91*** (10.8 g, 64%) as a white, crystalline solid, m.p. = 58-60 °C.

^{1}H NMR (300 MHz, d_6-DMSO) δ 5.80 (d, *J* = 2.4 Hz, 1H), 5.72 (br s, 1H), 5.52 (br s, 1H), 4.18 (dd, *J* = 8.4 and 2.4 Hz, 1H), 3.98 (d, *J* = 3.6 Hz, 1H), 3.91 (dd, *J* = 11.4 and 8.4 Hz, 1H), 3.56 (dd, *J* = 11.4 and 3.6 Hz, 1H) (one signal due to hydroxyl group signal not observed).

^{13}C NMR (75 MHz, d_6-DMSO) δ 132.6, 130.9, 72.0, 71.7, 70.3, 59.8.

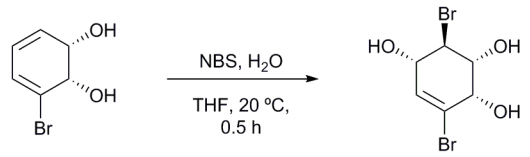
IR (KBr) ν_{max} 3316, 1655, 1445, 1330, 1241, 1193, 1092, 1061, 1035, 1008, 879 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/z* 244 and 242 ($M^{+\bullet}$, 2 and 1%), 226 and 224 (both 30), 209 and 207 (6 and 4), 191 and 189 (10 and 9), 163 (100), 145 (90), 20 (96), 91 (85), 81 (75), 53 (100).

HREIMS Found: $M^{+\bullet}$, 241.9336. $C_6H_8{}^{79}Br^{35}ClO_3$ requires $M^{+\bullet}$, 241.9345.

Optical rotation $[\alpha]_D$ −67.0 (*c* 5.0, THF).

(1*S*,2*R*,3*R*,4*S*)-3,6-dibromocyclohex-5-ene-1,2,4-triol (92)



89 **92**

Compound **89** (5.30 g, 27.8 mmol) was subjected to the same procedure as employed for the conversion **88** → **91** and an orange crystalline solid was thereby obtained. Subjection of this material to flash chromatography (silica, 1:1 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.3) then gave the *title compound **92*** (7.00 g, 89%) as a white, crystalline solid, m.p. = 116-118 °C.

^{1}H NMR (300 MHz, d_6-DMSO) δ 6.00 (d, *J* = 2.1 Hz, 1H), 5.79 (d, *J* = 7.2 Hz, 1H), 5.73 (d, *J* = 6.3 Hz, 1H), 5.47 (d, *J* = 7.8 Hz, 1H), 4.14 (m, 1H), 4.05 (m, 1H), 3.91 (m, 1H), 3.58 (m, 1H).

^{13}C NMR (75 MHz, d_6-DMSO) δ 135.0, 123.6, 73.3, 73.1, 70.5, 59.7.

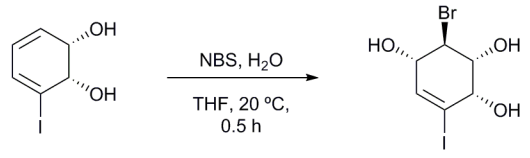
IR (KBr) ν_{max} 3271, 1641, 1471, 1277, 1193, 1095, 1065, 1024, 998, 894, 869, 749 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/*z 288 ($M^{+\bullet}$, <1%), 272, 270 and 268 (2, 5 and 2), 191 and 189 (90 and 85), 166 and 164 (87 and 86), 82 (85), 81 (95), 71 (87), 53 (100).

HREIMS Found: $M^{+\bullet}$, 287.8825. $C_6H_8{}^{79}Br^{81}BrO_3$ requires $M^{+\bullet}$, 287.8820.

Optical rotation $[\alpha]_D$ −61.7 (*c* 5.0, EtOH).

(1*S*,2*R*,3*R*,4*S*)-3-bromo-6-iodocyclohex-5-ene-1,2,4-triol (93)



90 **93**

Compound **90** (10.6 g, 44.6 mmol) was subjected to the same procedure as employed for the conversion **88** → **91** and a light-yellow oil was thereby obtained. Subjection of this material to flash chromatography (silica, 1:1 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.3) then gave the *title compound **93*** (9.84 g, 66%) as a white, crystalline solid, m.p. = 125-127 °C.

^{1}H NMR (300 MHz, d_6-DMSO) δ 6.22 (d, *J* = 2.4 Hz, 1H), 5.77 (d, *J* = 7.2 Hz, 1H), 5.66 (d, *J* = 6.3 Hz, 1H), 5.44 (d, *J* = 7.5 Hz, 1H), 4.09 (m, 2H), 3.91 (m, 1H), 3.58 (m, 1H).

^{13}C NMR (75 MHz, d_6-DMSO) δ 142.0, 100.4, 76.1, 74.5, 70.3, 59.9.

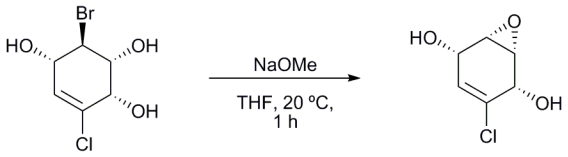
IR (KBr) ν_{max} 3255, 1629, 1281, 1191, 1106, 1065, 1021, 998, 890, 863 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/*z 336 and 334 ($M^{+\bullet}$, both 10%), 237 (100), 212 (95), 191 (45), 128 (35), 110 (75), 82 (60), 43 (60).

HREIMS Found: $M^{+\bullet}$, 333.8700. $C_6H_8{}^{79}Br^{127}IO_3$ requires $M^{+\bullet}$, 333.8702.

Optical rotation $[\alpha]_D$ −63.5 (*c* 5.0, THF).

(1R,2S,5S,6S)-3-chloro-7-oxabicyclo[4.1.0]hept-3-ene-2,5-diol (94)



91 **94**

A magnetically stirred solution of compound **91** (3.34g, 13.7 mmol) in THF (35 mL) was treated with finely-ground sodium methoxide (820 mg, 15.2 mmol). The ensuing mixture was stirred at 20 °C for 1 h and then passed through a plug of silica gel and concentrated under reduced pressure to afford a dark-brown oil. Subjection of this material to flash chromatography (silica, 3:1 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.3) then gave the *title compound **94*** (1.17 g, 52%) as a white, crystalline solid, m.p. = 140-142 °C.

^{1}H NMR (300 MHz, d_6-DMSO) δ 5.78 (d, *J* = 7.5 Hz, 1H), 5.53 (dd, *J* = 4.1, 2.0 Hz, 1H), 5.46 (d, *J* = 6.6 Hz, 1H), 4.38 (m, 1H), 4.24 (m, 1H) (signals due to hydroxyl group proton not observed).

^{13}C NMR (75 MHz, d_6-DMSO) δ 131.3, 126.3, 65.0, 64.8, 54.5, 54.1.

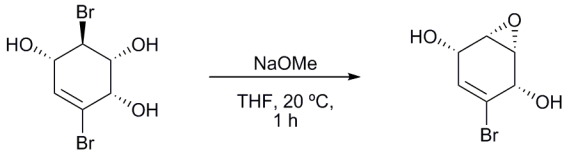
IR (KBr) ν_{max} 3246, 1654, 1409, 1351, 1280, 1255, 1045, 923, 811, 766, 661, 595 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/*z 164 and 162 ($M^{+\bullet}$, <1% and 3%), 127 (40), 115 (50), 109 (40), 81 (85), 71 (100), 53 (85), 41 (95).

HREIMS Found: $M^{+\bullet}$, 162.0086. $C_6H_7{}^{35}ClO_3$ requires $M^{+\bullet}$, 162.0084.

Optical rotation $[\alpha]_D$ +5.6 (*c* 5.0, THF).

(1R,2S,5S,6S)-3-bromo-7-oxabicyclo[4.1.0]hept-3-ene-2,5-diol (95)



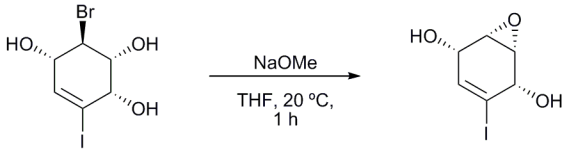
92 **95**

Compound **92** (1.69 g, 5.88 mmol) was subjected to the same procedure as employed for the conversion **91** → **94** and a dark-brown oil was thereby obtained. Subjection of this material to flash chromatography (silica, 3:1 v/v ethyl acetate/hexane elution) and concentration of the

appropriate fractions (R_f = 0.3) then gave the *title compound* ***95*** (0.670 g, 56%) as a white, crystalline solid, m.p. = 134-136 °C.

^{1}H NMR (300 MHz, d_6-DMSO) δ 5.80 (d, *J* = 7.8 Hz, 1H), 5.76 (d, *J* = 2.1 Hz, 1H), 5.48 (d, *J* = 6.6 Hz, 1H), 4.32 (m, 1H), 4.24 (m, 1H), 3.48-3.30 (complex m, 2H).
^{13}C NMR (75 MHz, d_6-DMSO) δ 130.4, 124.0, 65.8, 65.5, 54.4, 54.2.
IR (KBr) ν_{max} 3229, 1647, 1747, 1348, 1281, 1254, 1039, 943, 919, 882, 760 cm^{-1}.
Mass spectrum (EI, 70 eV) *m/*z 208 and 206 ($M^{+\bullet}$, both 15%), 191 and 189 (both 10), 161 and 159 (both 30), 127 (60), 81 (70), 71 (95), 54 (100).
HREIMS Found: $M^{+\bullet}$, 205.9579. $C_6H_7{}^{79}BrO_3$ requires $M^{+\bullet}$, 205.9579.
Optical rotation $[\alpha]_D$ +16.6 (*c* 5.0, THF).

(1*R*,2*S*,5*S*,6*S*)-3-iodo-7-oxabicyclo[4.1.0]hept-3-ene-2,5-diol (96)

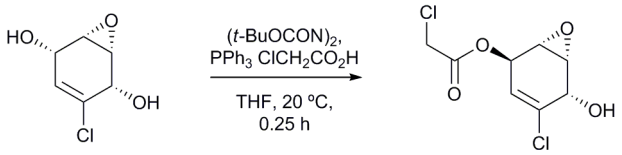


93 **96**

Compound **93** (9.84 g, 29.4 mmol) was subjected to the same procedure as employed for the conversion **91** → **94** and a dark-brown oil was thereby obtained. Subjection of this material to flash chromatography (silica, 3:1 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.3) then gave the *title compound* ***96*** (5.89 g, 79%) as a clear, crystalline solid, m.p. = 124-126 °C.

^{1}H NMR (300 MHz, d_6-DMSO) δ 6.06 (dd, *J* = 4.4 and 2.0 Hz, 1H), 5.83 (d, *J* = 8.1 Hz, 1H), 5.47 (d, *J* = 6.6 Hz, 1H), 4.26 (m, 1H), 4.13 (m, 1H) (signals due to hydroxyl group protons not observed).
^{13}C NMR (75 MHz, d_6-DMSO) δ 138.3, 103.7, 67.1, 66.2, 54.2, 53.5.
IR (KBr) ν_{max} 3232, 1630, 1282, 1050, 1035, 915, 874, 757, 651 cm^{-1}.
Mass spectrum (EI, 70 eV) *m/*z 254 ($M^{+\bullet}$, 100%), 207 (20), 127 (85), 109 (50), 81 (85), 71 (60), 53(99), 43(98).
HREIMS Found: $M^{+\bullet}$, 253.9442. $C_6H_7{}^{127}IO_3$ requires $M^{+\bullet}$, 253.9440.
Optical rotation $[\alpha]_D$ +26.4 (*c* 5.0, THF).

(1*S*,2*R*,5*S*,6*R*)-4-chloro-5-hydroxy-7-oxabicyclo[4.1.0]hept-3-en-2-yl 2-chloroacetate (98)

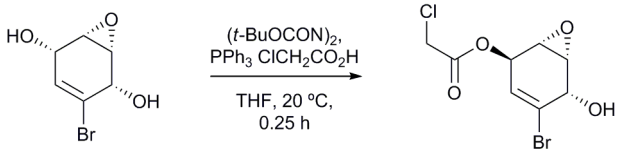


94 **98**

A magnetically stirred solution of compound **94** (1.41 g, 8.69 mmol) in THF (30 mL) was treated with chloroacetic acid (1.01 g, 10.7 mmol), di*tert*butyl azodicarboxylate (2.02 g, 8.78 mmol) and triphenylphosphine (2.28 g, 8.69 mmol). Then ensuing mixture was stirred at 20 °C for 0.25 h and then concentrated under reduced pressure to an amber-colored oil. Subjection of this material to flash chromatography (silica, 1:2 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.3) then gave the *title compound **98*** (1.50 g, 72%) as a white solid, m.p. = 78-81 °C.

^{1}H NMR (300 MHz, d_6-DMSO) δ 5.88 (ddd, *J* = 5.1, 1.7 and 1.7 Hz, 1H), 5.61 (ddd, *J* = 5.1, 2.4, 1.7 Hz, 1H), 4.51 (m, 1H), 4.10 (s, 2H), 3.68 (m, 1H), 3.50 (m, 1H), 2.78 (broad s, 1H).
^{13}C NMR (75 MHz, d_6-DMSO) δ 166.4, 138.0, 120.1, 67.1, 66.2, 54.4, 53.2, 40.5.
IR (KBr) ν_{max} 3449, 1753, 1656, 1402, 1324, 1298, 1188, 963, 930, 895, 832 cm^{-1}.
Mass spectrum (EI, 70 eV) *m/*z 238 ($M^{+\bullet}$, 1%), 162 (40), 146 and 144 (46 and 95), 81 (95), 77 (100), 71 (80), 53(90).
HREIMS Found: $M^{+\bullet}$, 237.9799. $C_8H_8{}^{35}Cl_2O_4$ requires $M^{+\bullet}$, 237.9800.
Optical rotation $[\alpha]_D$ −147.6 (*c* 5.0, $CHCl_3$).

(1*S*,2*R*,5*S*,6*R*)-4-bromo-5-hydroxy-7-oxabicyclo[4.1.0]hept-3-en-2-yl 2-chloroacetate (99)



95 **99**

Compound **95** (1.90 g, 9.19 mmol) was subjected to the same procedure as employed for the conversion **94** → **98** and an amber-colored oil was thereby obtained. Subjection of this material to flash chromatography (silica, 1:2 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.3) then gave the *title compound **99*** (1.52 g, 59%) as a white solid, m.p. = 99-102 °C.

^{1}H NMR (300 MHz, d_6-DMSO) δ 6.13 (m, 1H), 5.51 (m, 1H), 4.52 (m, 1H), 4.10 (s, 2H), 3.66 (mz, 1H), 3.50 (m, 1H), 2.75 (broad s, 1H).

^{13}C NMR (75 MHz, d_6-DMSO) δ 166.4, 130.3, 124.2, 67.63, 67.1, 54.3, 53.3, 40.5.

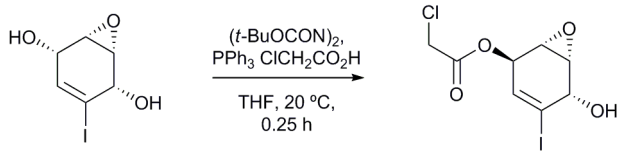
IR (KBr) ν_{max} 3241, 1750, 1631, 1407, 1325, 1296, 1169, 1057, 989, 949, 897, 830, 787 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/*z 208 and 206 [$(M-ClCHCO)^{+\bullet}$, both 10%], 190 and 188 (both 50), 127 (50), 109 (80), 81 (100).

HREIMS Found: $(M-ClCHCO)^{+\bullet}$, 207.9557. $C_8H_8{}^{79}BrClO_4$ requires $(M-ClCHCO)^{+\bullet}$, 207.9558.

Optical rotation $[\alpha]_D$ −145.8 (*c* 3.5, $CHCl_3$).

(1*S*,2*R*,5*S*,6*R*)-5-hydroxy-4-iodo-7-oxabicyclo[4.1.0]hept-3-en-2-yl 2-chloroacetate (100)



96 **100**

Compound **96** (1.92 g, 7.56 mmol) was subjected to the same procedure as employed for the conversion **94** → **98** and a amber-colored oil was thereby obtained. Subjection of this material to flash chromatography (silica, 1:2 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.3) then gave the *title compound **100*** (1.92 g, 77%) as a white, crystalline solid, m.p. = 112-114 °C.

^{1}H NMR (300 MHz, d_6-DMSO) δ 6.45 (m, 1H), 5.37 (ddd, *J* = 4.8, 2.4 and 1.5 Hz, 1H), 4.44 (m, 1H), 4.11 (s, 2H), 3.61 (m, 1H), 3.52 (m, 1H), 2.80 (broad s, 1H).

^{13}C NMR (75 MHz, d_6-DMSO) δ 166.4, 132.2, 109.0, 68.7, 67.3, 53.5, 53.4, 40.5.

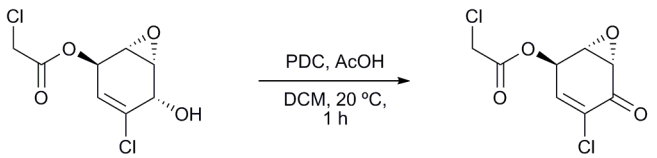
IR (KBr) ν_{max} 3241, 1750, 1631, 1407, 1325, 1296, 1169, 1057, 989, 949, 897, 830, 787 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/*z 332 and 330 ($M^{+\bullet}$, 3 and 10%), 236 (95), 206 (18), 127 (70), 109 (85), 81 (100), 76 (83).

HREIMS Found: $M^{+\bullet}$, 329.9161. $C_8H_8{}^{35}Cl^{127}IO_4$ requires $M^{+\bullet}$, 329.9156.

Optical rotation $[\alpha]_D$ −72.5 (*c* 5.0, $CHCl_3$).

(1*S*,2*R*,6*S*)-4-chloro-5-oxo-7-oxabicyclo[4.1.0]hept-3-en-2-yl 2-chloroacetate (101)



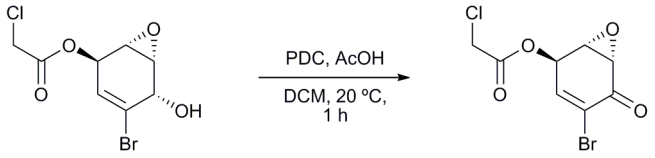
98 **101**

A magnetically stirred solution of compound **98** (481 mg, 2.01 mmol) in DCM (45 mL) was treated with acetic acid (0.14 mL, 2.45 mmol) and pyridinium dichromate (835 mg, 2.22 mmol).

The ensuing mixture was stirred at 20 °C for 1 h and then diluted with diethyl ether and passed through a plug of silica gel. The filtrate was concentrated under reduced pressure to a dark-brown oil. Subjection of this material to flash chromatography (silica, 1:4 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.3) then gave the *title compound* ***101*** (310 mg, 65%) as a white solid, m.p. 84-85 °C.

^{1}H NMR (300 MHz, d_6-DMSO) δ 6.78 (dd, *J* = 5.4 and 2.4 Hz, 1H), 5.87 (ddd, *J* = 5.4, 1.5 and 1.5 Hz, 1H), 4.13 (s, 2H), 3.80 (m, 1H), 3.68 (m, 1H).
^{13}C NMR (75 MHz, d_6-DMSO) δ 185.4, 166.2, 134.3, 134.0, 66.2, 54.4, 53.2, 40.2.
IR (KBr) ν_{max} 1767, 1703, 1317, 1157, 975, 787 cm^{-1}.
Mass spectrum (EI, 70 eV) *m/*z 238 and 236 ($M^{+\bullet}$, 3 and 5%), 162 and 160 (30 and 65), 131 (80), 115 (52), 76 and 77 (60 and 100), 51 (90).
HREIMS Found: $M^{+\bullet}$, 235.9643. $C_8H_6{}^{35}Cl_2O_4$ requires $M^{+\bullet}$, 235.9643.
Optical rotation $[\alpha]_D$ −327.3 (*c* 5.0, $CHCl_3$).

(1S,2R,6S)-4-bromo-5-oxo-7-oxabicyclo[4.1.0]hept-3-en-2-yl 2-chloroacetate (102)

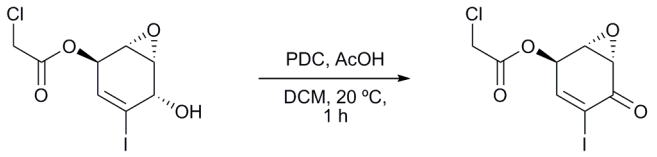


99 **102**

Compound **99** (518 mg, 1.83 mmol) was subjected to the same procedure as employed for the conversion **98** → **101** and a dark-brown oil was thereby obtained. Subjection of this material to flash chromatography (silica, 1:4 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.3) then gave the *title compound* ***102*** (394 mg, 77%) as a pale-yellow oil.

^{1}H NMR (300 MHz, d_6-DMSO) δ 7.04 (dd, *J* = 5.4 and 2.4 Hz, 1H), 5.78 (ddd, *J* = 5.4, 1.2 and 1.2 Hz, 1H), 4.13 (s, 2H), 3.80 (m, 1H), 3.68 (m, 1H).
^{13}C NMR (75 MHz, d_6-DMSO) δ 185.5, 166.2, 138.3, 126.0, 66.6, 54.4, 52.7, 40.2.
IR (KBr) ν_{max} 1767, 1703, 1317, 1157, 975, 787 cm^{-1}.
Mass spectrum (EI, 70 eV) *m/*z 284, 282 and 280 ($M^{+\bullet}$, 2, 10 and 8%), 206 and 204 (both 75), 177 and 175 (both 70), 97 (60), 79 (87), 77 (95), 51 (100).
HREIMS Found: $M^{+\bullet}$, 279.9139. $C_8H_6{}^{79}Br^{35}ClO_4$ requires $M^{+\bullet}$, 279.9138.
Optical rotation $[\alpha]_D$ −251.3 (*c* 5.0, $CHCl_3$).

(1S,2R,6S)-4-iodo-5-oxo-7-oxabicyclo[4.1.0]hept-3-en-2-yl 2-chloroacetate (103)



100 **103**

Compound **100** (3.45 g, 10.4 mmol) was subjected to the same procedure as employed for the conversion **98** → **101** and a dark-brown oil was thereby obtained. Subjection of this material to flash chromatography (silica, 1:4 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.3) then gave the *title compound **103*** (2.57 g, 75%) as a pale-yellow oil.

^{1}H NMR (300 MHz, d_6-DMSO) δ 7.37 (dd, *J* = 5.4 and 2.4 Hz, 1H), 5.69 (ddd, *J* = 5.4, 1.2 and 1.2 Hz, 1H), 4.14 (s, 2H), 3.82 (m, 1H), 3.68 (dd, *J* = 3.6 and 1.2 Hz, 1H).
^{13}C NMR (75 MHz, d_6-DMSO) δ 186.9, 166.2, 146.1, 105.6, 67.2, 54.5, 51.1, 40.2.
IR (KBr) ν_{max} 1767, 1697, 1318, 1157, 1003, 969, 781 cm^{-1}.
Mass spectrum (EI, 70 eV) *m*/z 330 and 328 ($M^{+\bullet}$, 25 and 60%), 252 (100), 223 (63), 125 (66), 125 (66), 77 (85).
HREIMS Found: $M^{+\bullet}$, 327.8996. $C_8H_6{}^{35}ClIO_4$ requires $M^{+\bullet}$, 327.8999.
Optical rotation $[\alpha]_D$ −140.2 (*c* 3.2 acetone).

(1*S*,5*R*,6*S*)-3-chloro-5-hydroxy-7-oxabicyclo[4.1.0]hept-3-en-2-one (104)

101 **104**

A magnetically stirred solution of compound **101** (295 mg, 1.24 mmol) in methanol (10 mL) was treated with zinc(II) acetate dihydrate (600 mg, 2.73 mmol). The ensuing mixture was stirred at 20 °C for 1 h and then concentrated under reduced pressure to afford a white solid. This material was treated with ethyl acetate (20 mL) and the resulting mixture washed with water (1 × 20 mL) and brine (1 × 20 mL) before being dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a white solid. Subjection of this material to flash chromatography (silica, 2:3 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.35) then gave the title compound **104** (168 mg, 84%) as a white, crystalline solid, m.p. = 127-128 °C.

^{1}H NMR (300 MHz, d_6-acetone) δ 7.00 (dd, *J* = 5.1, 2.1 Hz, 1H), 5.17 (br s, 1H), 4.79 (br d, *J* = 5.4 Hz, 1H), 3.86 (ddd, *J* = 3.6, 2.1, 1.2 Hz, 1H), 3.67 (dd, *J* = 3.6, 1.2 Hz, 1H).

^{13}C NMR (75 MHz, d_6-acetone) δ 206.4, 187.4, 142.4, 130.9, 64.3, 58.5, 54.5.

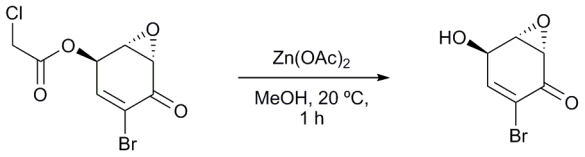
IR (KBr) ν_{max} 3494, 1686, 1613, 1391, 1041, 1030, 986, 798 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/*z 160 and162 ($M^{+\bullet}$, 11 and 4%), 131 and 133 (70 and 33), 125 (100), 103 and 105 (88 and 41), 97 (99), 71 (100), 39 (69).

HREIMS Found: $M^{+\bullet}$, 159.9927. $C_6H_5{}^{35}ClO_3$ requires $M^{+\bullet}$, 159.9927.

Optical rotation $[\alpha]_D$ −290.5 (*c* 3.45, acetone).

Compound *ent*-55 [(−)-bromoxone]



102 **55**

A magnetically stirred solution of compound **102** (132 mg, 0.469 mmol) in methanol (10 mL) was treated with zinc(II) acetate dihydrate (245 mg, 1.12 mmol). The ensuing mixture was stirred at 20 °C for 1 h and then concentrated under reduced pressure to afford a white solid. This material was treated with ethyl acetate (20 mL) and the resulting mixture washed with water (1 × 20 mL) and brine (1 × 20 mL) before being dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a white solid. Subjection of this material to flash chromatography (silica, 2:3 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.35) then gave the title compound *ent*-**55** (850 mg, 88%) as a white, crystalline solid, m.p. = 131-134 °C.

^{1}H NMR (300 MHz, d_6-acetone) δ 7.23 (dd, *J* = 5.1 and 2.1 Hz, 1H), 5.17 (broad s, 1H), 4.69 (broad d, *J* = 4.2 Hz, 1H), 3.83 (m, 1H), 3.63 (m, 1H).

^{13}C NMR (75 MHz, d_6-acetone) δ 187.4, 146.8, 122.5, 65.0, 58.5, 54.0.

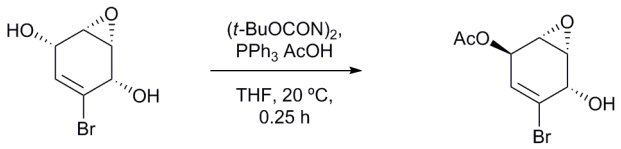
IR (KBr) ν_{max} 3467, 1689, 1611, 1254, 1046, 975, 896, 867, 791, 613, 545 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/*z 206 and 204 ($M^{+\bullet}$, 15%), 177 and 175 (both 30), 149 and 147 (both 30), 125 (100), 97 (62), 71 (60), 39 (55).

HREIMS Found: $M^{+\bullet}$, 203.9424. $C_8H_5{}^{79}BrO_3$ requires $M^{+\bullet}$, 203.9422.

Optical rotation $[\alpha]_D$ −187.1 (*c* 1.75, acetone).

(1*S*,2*R*,5*S*,6*R*)-4-bromo-5-hydroxy-7-oxabicyclo[4.1.0]hept-3-en-2-yl acetate (107)

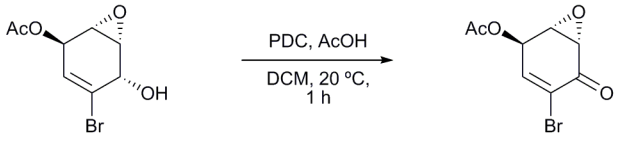


95 **107**

A magnetically stirred solution of compound **95** (300 mg, 1.45 mmol) in THF (5 mL) was treated with acetic acid (125 µL, 2.19 mmol), di*tert*butyl azodicarboxylate (337 mg, 1.46 mmol) and triphenylphosphine (387 mg, 1.48 mmol). The ensuing mixture was stirred at 20 °C for 0.25 h and then concentrated under reduced pressure to an amber-colored oil. Subjection of this material to flash chromatography (silica, 1:2 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.3) then gave the *title compound* ***107*** (316 mg, 88%) as a white solid, m.p. = 75-77 °C.

^{1}H NMR (300 MHz, $CHCl_3$) δ 6.11 (ddd, *J* = 4.5, 1.8, 1.8 Hz, 1H), 5.44 (dm, 1H), 4.50 (m, 1H), 3.63 (dm, *J* = 9.9 Hz, 1H), 3.46 (m, 1H), 2.80 (d, *J* = 9.9 Hz, 1H), 2.09 (s, 3H) (signal due to the hydroxyl proton not observed).
^{13}C NMR (75 MHz, $CHCl_3$) δ 169.9, 129.2, 125.2, 67.2, 65.5, 54.3, 53.7, 20.7.
IR (KBr) ν_{max} 3457, 2928, 1737, 1648, 1372, 1231, 1060, 1024, 893, 610 cm^{-1}.
Mass spectrum (EI, 70 eV) *m/*z 250 and 248 ($M^{+\bullet}$, both 3%), 208 and 206 (both 40), 190 and 188 (both 49), 127 (61), 109 (69), 81 (81), 43 (100).
HREIMS Found: $M^{+\bullet}$, 247.9684. $C_8H_9BrO_4$ requires $M^{+\bullet}$, 247.9684.
Optical rotation $[\alpha]_D$ −127.8 (*c* 5.0, $CHCl_3$).

Compound 106 [(−)-*O*-acetylbromoxone]



107 **106**

A magnetically stirred solution of compound **107** (118 mg, 0.47 mmol) in DCM (10 mL) was treated with acetic acid (40 µL, 0.63 mmol) and PDC (235 mg, 0.62 mmol). The ensuing mixture was stirred at 20 °C for 1.5 h and then treated with silica gel (5 mL) and diethyl ether (10 mL). The resulting mixture was filtered through Celite™ and the filtrate concentrated under reduced pressure to give a pale-orange oil. Subjection of this material to flash chromatography (silica, 1:3 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.3) then gave the *title compound* ent-***106*** (60 mg, 51%) as a white, crystalline solid, m.p. = 93.0-93.2 °C [lit.[105] m.p. (for **106**) = 93-94 °C].

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 (dd, *J* = 5.4 and 2.4 Hz, 1H), 5.73 (ddd, *J* = 5.4, 1.2 and 1.2 Hz, 1H), 3.77 (m, 1H), 3.68 (dd, *J* = 3.6 and 1.2 Hz, 1H), 2.14 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 185.9, 169.5, 139.6, 125.4, 65.2, 54.9, 52.9, 20.5.

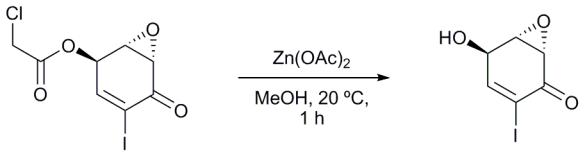
IR (KBr) ν_{max} 1742, 1704, 1614, 1371, 1225, 1030, 971, 787 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/*z 248 and 246 ($M^{+\bullet}$, both 5%), 206 and 204 (both 61), 177 and 175 (both 43), 97 (62), 51 (59), 43 (100).

HREIMS Found $M^{+\bullet}$, 245.9535. $C_8H_7{}^{79}BrO_4$ requires $M^{+\bullet}$, 245.9528.

Optical rotation $[\alpha]_D$ −278.2 (c 2.55, $CHCl_3$) {lit.[105] $[\alpha]_D$ (for **1**) +265 (*c* 0.12, $CHCl_3$)}.

(1*S*,5*R*,6*S*)-5-hydroxy-3-iodo-7-oxabicyclo[4.1.0]hept-3-en-2-one (105)



103 **105**

Compound **103** (152 mg, 0.463 mmol) was subjected to the same procedure as employed for the conversion **102** → *ent*-**55** and a white solid was thereby obtained. Subjection of this material to flash chromatography (silica, 2:3 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.35) then gave the *title compound **105*** (101 mg, 87%) as a clear, crystalline solid, m.p. = 131-133 °C.

^{1}H NMR (300 MHz, d_6-acetone) δ 7.58 (dd, *J* = 5.4 and 2.4 Hz, 1H), 5.20 (d, J = 8.7 Hz, 1H), 4.61 (m, 1H), 3.87 (m, 1H), 3.63 (dd, *J* = 3.6 and 1.5 Hz, 1H).

^{13}C NMR (75 MHz, d_6-acetone) δ 188.9, 155.0, 101.7, 65.8, 58.6, 52.4.

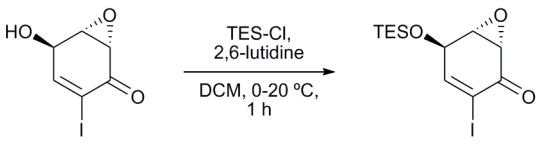
IR (KBr) ν_{max} 3480, 3049, 1678, 1595, 1246, 1043, 865, 786, 598, 538 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/*z 252 ($M^{+\bullet}$, 70%), 223 (20), 125 (100), 97 (50), 71 (40), 53 (40), 39 (42).

HREIMS Found: $M^{+\bullet}$, 251.9283. $C_6H_5{}^{127}IO_3$ requires $M^{+\bullet}$, 251.9283.

Optical rotation $[\alpha]_D$ −102.1 (*c* 1.05, acetone).

(1*S*,5*R*,6*S*)-5-triethylsilyloxy-3-iodo-7-oxabicyclo[4.1.0]hept-3-en-2-one (146)



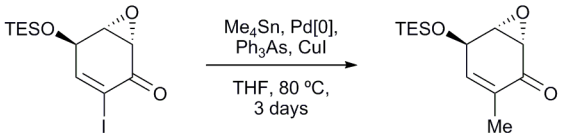
105 **146**

A magnetically stirred solution of compound **105** (1.52 g, 6.04 mmol) in DCM (25 mL) maintained at 0 °C was treated with 2,6-lutidine (1.05 mL, 8.94 mmol) and chlorotriethylsilane

(1.50 mL, 8.94 mmol). The ensuing mixture was stirred for 0.5 h and ethyl acetate (25 mL) was added and the solution was washed with NH_4Cl (25 mL of a saturated aqueous solution) and brine (25 mL of a saturated aqueous solution). The separated organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure to yield a pale-yellow oil. Subjection of this material to flash chromatography (silica, 2:50 v/v diethyl ether/hexane elution) and concentration of the appropriate fractions (R_f = 0.2) then gave the *title compound* ***146*** (1.21 g, 55%) as a pale-yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (dd, *J* = 5.4 and 2.4 Hz, 1H), 4.57 (m, 1H), 3.69 (m, 1H), 3.61 (dd, *J* = 3.6 and 1.2 Hz, 1H), 0.98 (t, *J* = 8.1 Hz, 9H), 0.67 (q, *J* = 8.1 Hz, 6H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 187.5, 152.6, 102.0, 65.8, 58.2, 51.7, 6.6, 4.7.
IR (KBr) ν_{max} 2956, 2877, 1696, 1091, 789, 748 cm^{-1}.
Mass spectrum (EI, 70 eV) *m/*z 366 ($M^{+\bullet}$, 25%), 337 (70), 210 (100), 182 (60).
HREIMS Found: $M^{+\bullet}$, 366.0157. $C_{12}H_{19}{}^{127}IO_3Si$ requires $M^{+\bullet}$, 366.0148.
Optical rotation $[\alpha]_D$ −107.7 (*c* 1.65, $CHCl_3$).

(1S,5R,6R)-3-methyl-5-(triethylsilyloxy)-7-oxabicyclo[4.1.0]hept-3-en-2-one (147)



146 **147**

A magnetically stirred solution of compound **146** (150 mg, 0.410 mmol) and tetramethyl stannane (225 mg, 1.26 mmol) in THF was treated with palladium(II) acetate (6 mg, 27 μmol), copper(I) iodide (7 mg, 37 μmol) and triphenylarsine (24 mg, 78 μmol). The ensuing mixture was heated at 80 °C in a sealed tube for 72 h, cooled and diluted with Na_2SO_3 (5 mL of a 10% w/w aqueous solution) and KF (5 mL of a 10% w/w aqueous solution) and extracted with diethyl ether (3 × 10 mL). The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford a pale-yellow oil. Subjection of this material to flash chromatography (silica, 2:50 v/v diethyl ether/hexane elution) and concentration of the appropriate fractions (R_f = 0.2) then gave the *title compound* ***147*** (39 mg, 37%) as a clear oil.

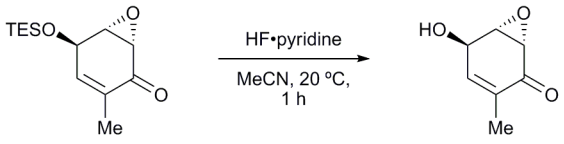
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.29 (m, 1H), 4.62 (m, 1H), 3.64 (m, 1H), 3.48 (dd, *J* = 3.6 and 1.2 Hz, 1H), 1.82 (broadened s, 3H), 0.99 (t, *J* = 8.1 Hz, 9H), 0.67 (q, *J* = 8.1 Hz, 6H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 193.9, 139.5, 133.4, 63.7, 58.4, 53.4, 15.9, 6.7, 4.8.
IR (KBr) ν_{max} 2957, 1685, 1092, 1074, 1005, 825, 747, 745 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/*z 254 ($M^{+\bullet}$, 10%), 225 (100), 197 (90), 103 (80), 95 (77), 87 (55), 75 (85), 59 (65).

HREIMS Found: $M^{+\bullet}$, 254.1332. $C_{13}H_{22}O_3Si$ requires $M^{+\bullet}$, 254.1338.

Optical rotation $[\alpha]_D$ −192.7 (*c* 0.95, Et_2O).

Compound *ent*-108 [(−)-Epiepoformin]



147 *ent*-**108**

A magnetically stirred solution of compound **147** (28 mg, 0.11 mmol) in acetonitrile (1.2 mL) was treated with hydrogen fluoride pyridine (200 μL, hydrogen fluoride, ~70%, pyridine, ~30%). The ensuing mixture was stirred at 20 °C for 1 h and then diluted with $NaHCO_3$ (5 mL of a saturated aqueous solution) and extracted with ethyl acetate (3 × 5 mL). The combined organic extracts were washed with water and brine and then concentrated under reduced pressure to yield a pale-yellow oil. Subjection of this material to flash chromatography (silica, 1:2 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.35) then gave the *title compound* ent-***108*** (12 mg, 78%) as a white, crystalline solid, m.p. = 85-85 °C.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.46 (m, 1H), 4.66 (broad m, 1H), 3.79 (m, 1H), 3.51 (dd, *J* = 3.6 and 1.2 Hz, 1H), 2.20 (broad d, *J* = 7.8 Hz, 1H), 1.84 (broadened s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 194.5, 139.1, 134.3, 63.2, 57.7, 53.4, 15.9.

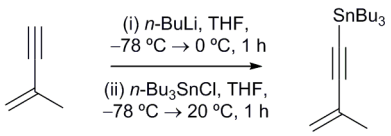
IR (KBr) ν_{max} 3420, 2925, 1670, 1040, 825 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/*z 140 ($M^{+\bullet}$, 20%), 111 (100), 97 (60), 83 (50), 71 (68), 69 (75), 55 (60), 39 (60).

HREIMS Found: $M^{+\bullet}$, 140.0471. $C_7H_8O_3$ requires $M^{+\bullet}$, 140.0473.

Optical rotation $[\alpha]_D$ −311.0 (*c* 0.27, EtOH) {lit.[85] $[\alpha]_D$ (for **108**) +316.04 (*c* 0.37, EtOH)}.

tributyl(3-methylbut-3-en-1-ynyl)stannane (156)



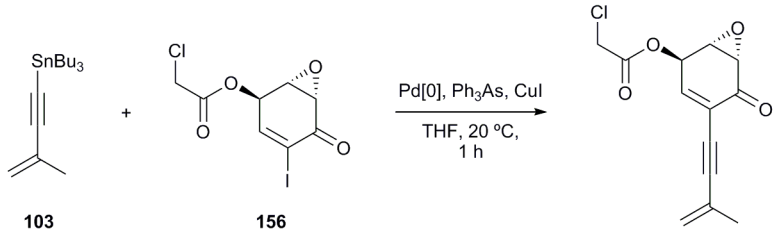
245 **156**

A magnetically stirred solution of 2-methylbut-1-en-3-yne (0.690 g, 10.4 mmol, ex. Aldrich) in THF (30 mL) was cooled to −78 °C then treated with n-BuLi (7.5 mL of a 1.6 M solution in

hexanes, 12.0 mmol). The ensuing mixture was warmed to 0 °C over 1 h and then cooled to −78 °C and treated with tri-*n*-butyltin chloride (3.1 mL, 11.4 mmol) before being warmed to 20 °C over 1 h and then filtered through a pad of TLC-grade silica gel. The filtrate thus obtained was concentrated under reduced pressure to afford the title compound **156**[89] (3.65 g, 99%) as a clear, colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.28 (m, 1H), 5.16 (m, 1H), 1.89 (m, 3H), 1.55 (m, 6H), 1.34 (m, 6H), 1.00 (m, 6H), 0.90 (t, *J* = 7.4 Hz, 9H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 127.4, 121.4, 111.4, 91.9, 28.7, 26.9, 23.8, 13.6, 11.1.
IR (KBr) ν_{max} 2956, 2923, 2872, 2853, 2129, 1610, 1464, 1376, 1270, 1073, 965, 891 cm^{-1}.
Mass spectrum (EI, 70 eV) *m/*z 355 ($M^{+\bullet}$, 5%), 299 (100), 269 (10), 243 (30), 185 (50).
HREIMS Found: $(M–Bu^{\bullet})^+$, 299.0822. $C_{13}H_{23}{}^{120}Sn$ requires $(M−Bu^{\bullet})^+$, 299.0822.

(1*S*,2*R*,6*S*)-4-(3-methylbut-3-en-1-ynyl)-5-oxo-7-oxabicyclo[4.1.0]hept-3-en-2-yl 2-chloroacetate (173)



103 **156** **173**

A magnetically stirred solution of compound **156** (507 mg, 1.54 mmol) and compound **103** (842 mg, 2.37 mmol) in THF (15 mL) was treated with palladium(II) acetate (20 mg, 89 μmol), copper(I) iodide (20 mg, 105 μmol) and triphenylarsine (85 mg, 280 μmol). The ensuing mixture was stirred at 20 °C for 1 h, cooled and brine (30mL of a saturated aqueous solution) was added. The mixture was extracted with diethyl ether (2 × 30 mL) and the combined organic extracts were concentrated under reduced pressure to afford a dark-brown oil. Subjection of this material to flash chromatography (silica, 1:4 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.3) then gave the *title compound **173*** (252 mg, 61%) as a pale-yellow oil.

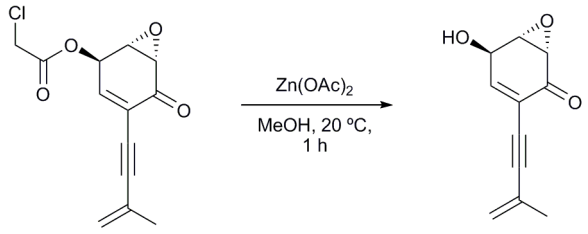
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.75 (dd, *J* = 4.8 and 2.4 Hz, 1H), 5.87 (ddd, *J* = 4.8, 1.2 and 1.2 Hz, 1H), 5.41 (broad s, 1H), 5.34 (m, 1H), 4.12 (m, 2H), 3.78 (m, 1H), 3.61 (broad d, *J* = 3.6 Hz, 1H), 1.91 (broadened s, 3H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 189.1, 166.3, 139.1, 125.7, 125.5, 124.5, 97.0, 80.8, 65.8, 54.2, 52.8, 40.3, 22.9.
IR (KBr) ν_{max} 1767, 1701, 1163, 991 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/*z 268 and 266 ($M^{+\bullet}$, 25 and 60%), 190 (70), 162 (70), 161 (65), 77 (100).

HREIMS Found: $M^{+\bullet}$, 266.0346. $C_{13}H_{11}{}^{35}ClO_4$ requires $M^{+\bullet}$, 266.0346.

Optical rotation $[\alpha]_D$ −278.8 (c 2.75, $CHCl_3$).

Compound 148 [(−)-harveynone]



173 **148**

A magnetically stirred solution of compound **173** (244 mg, 0.915 mmol) in methanol (20 mL) was treated with zinc(II) acetate dihydrate (402 mg, 1.83 mmol). The ensuing mixture was stirred at 20 °C for 1 h and then diluted with diethyl ether (50 mL) and washed with brine (3 × 30 mL). The separated organic layer was dried ($MgSO_4$), filtere and concentrated under reduced pressure to deliver a brown oil. Subjection of this material to flash chromatography (silica, 1:2 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.2) then gave the title compound **148**[90,92] (133 mg, 76%) as a pale-yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.85 (dd, *J* = 5.4 and 2.7 Hz, 1H), 5.43 (m, 1H), 5.35 (m, 1H), 4.73 (broad m, 1H), 3.85 (m, 1H), 3.55 (dd, *J* = 3.6 and 1.2 Hz, 1H), 3.00 (broad d, *J* = 9.3 Hz, 1H), 1.93 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 191.5, 146.1, 125.9, 124.1, 122.7, 95.9, 81.2, 63.2, 57.6, 53.5, 23.0.

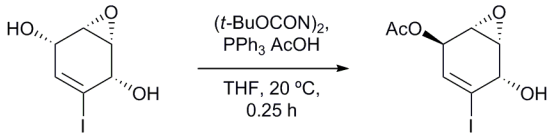
IR (KBr) ν_{max} 3233, 1689, 1615, 1437, 1235, 1031, 850 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/*z 190 ($M^{+\bullet}$, 100%), 162 (70), 161 (85), 133 (60), 91 (80), 83 (80).

HREIMS Found: $M^{+\bullet}$, 190.0630. $C_{11}H_{10}O_3$ requires $M^{+\bullet}$, 190.0630.

Optical rotation $[\alpha]_D$ −204.9 0 (*c* 0.89, MeOH) {lit.[92] $[\alpha]_D$ −207.4 (c 0.45, MeOH)}.

(1*S*,2*R*,5*S*,6*R*)-5-hydroxy-4-iodo-7-oxabicyclo[4.1.0]hept-3-en-2-yl acetate (188)

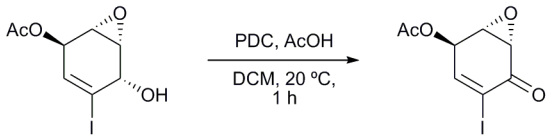


96 **188**

A magnetically stirred solution of compound **96** (962 mg, 3.79 mmol) in THF (15 mL) was treated with acetic acid (330 µL, 5.77 mmol), di*tert*butyl azodicarboxylate (889 mg, 3.86 mmol) and triphenylphosphine (997 mg, 3.80 mmol). The ensuing mixture was stirred at 20 °C for 0.25 h and then concentrated under reduced pressure to give an amber-colored oil. Subjection of this material to flash chromatography (silica, 1:2 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.3) then gave the *title compound **188*** (630 mg, 56%) as a white solid, m.p. = 101-104 °C.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.40 (ddd, *J* = 4.8, 1.8, 0.9 Hz, 1H), 5.30 (dm, *J* = 4.8 Hz, 1H), 4.40 (m, 1H), 3.56 (m, 1H), 3.45 (m, 1H), 3.01 (broad s, 1H), 2.08 (s, 3H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 169.8, 133.1, 108.0, 68.6, 65.6, 53.7, 53.5, 20.7.
IR (KBr) ν_{max} 3468, 1727, 1375, 1236, 1064, 1022, 890 cm^{-1}.
Mass spectrum (EI, 70 eV) *m*/z 296 ($M^{+\bullet}$, 9%), 254 (71), 236 (94), 127 (100), 109 (96), 81 (98), 43 (99).
HREIMS Found: $M^{+\bullet}$, 295.9541. $C_8H_9{}^{127}IO_4$ requires $M^{+\bullet}$, 295.9546.
Optical rotation $[\alpha]_D$ −92.2 (*c* 5.0, $CHCl_3$).

(1*S*,2*R*,6*S*)-4-iodo-5-oxo-7-oxabicyclo[4.1.0]hept-3-en-2-yl acetate (189)



188 **189**

A magnetically stirred solution of compound **188** (587 mg, 1.98 mmol) in DCM (40 mL) was treated with acetic acid (140 µL, 2.45 mmol) and pyridinium dichromate (834 mg, 2.22 mmol). The ensuing mixture was stirred at 20 °C for 5 h and then diluted with diethyl ether (40 mL) and passed through a pad of TLC-grade silica gel. The filtrate was concentrated under reduced pressure to a dark-brown oil that was subjected to flash chromatography (silica, 1:3 v/v ethyl acetate/hexane elution). Concentration of the appropriate fractions (R_f = 0.3) then gave the *title compound **189*** (445 mg, 76%) as a white solid m.p. = 115-118 °C.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 (dd, *J* = 5.4 and 2.4 Hz, 1H), 5.62 (ddd, *J* = 5.4, 1.2, 1.2 Hz, 1H), 3.77 (m, 1H), 3.65 (dd, *J* = 3.3 and 1.2 Hz, 1H), 2.13 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 187.2, 169.4, 147.5, 104.7, 65.7, 55.0, 51.2, 20.5.

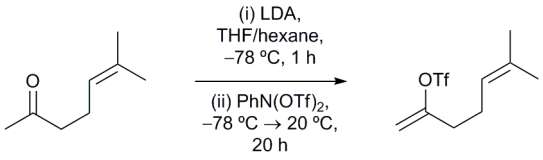
IR (KBr) ν_{max} 2915, 1743, 1695, 1605, 1374, 1334, 1241, 1216, 1031, 908, 872, 805, 780, 588, 544 cm^{-1}.

Mass spectrum (EI, 70 eV) *m*/z 294 ($M^{+\bullet}$, 25%), 252 (72), 223 (23), 125 (38), 43 (100).

HREIMS Found $M^{+\bullet}$, 293.9381. $C_8H_7{}^{127}IO_4$ requires $M^{+\bullet}$, 293.9389.

Optical rotation $[\alpha]_D$ −169.9 (*c* 5.0, acetone).

6-methylhepta-1,5-dien-2-yl trifluoromethanesulfonate (193)



192 **193**

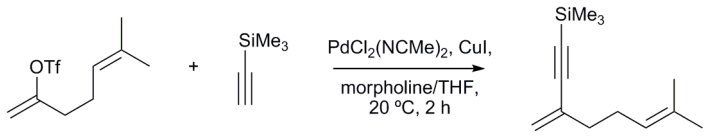
A magnetically stirred solution of di*iso*propylamine (7.50 mL, 53.5 mmol) in THF (45 mL) maintained at 0 °C was treated with *n*-butyllithium (34.0 mL of a 1.6 M solution in hexane, 54.4 mmol). After 0.25 h the reaction mixture was warmed to 20 °C, kept at this temperature for 0.25 h then cooled to −78 °C before being treated, dropwise, with ketone **192** (6.1 mL, 41.4 mmol, *ex* Aldrich). Stirring was continued for 1 h and then phenyl triflimide (15.5 g, 43.5 mmol, *ex* Aldrich) was added to the reaction mixture. This was stirred for a further 20 h during which time it was allowed to warm to 20 °C. The reaction mixture was then diluted with hexane (100 mL) and washed with water (2 × 100 mL) and brine (1 × 100 mL) before being dried ($MgSO_4$), filtered and concentrated under reduced pressure to a yellow oil. Subjection of this material to flash chromatography (silica, hexane elution) and concentration of the appropriate fractions (R_f = 0.2) gave enol triflate **193**[90,101,102] (7.14 g, 67%) as a pale yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.09 (d, *J* = 3.6 Hz, 1H), 5.06 (dm, *J* = 7.2 Hz, 1H), 4.93 (broad d, *J* = 3.6 Hz, 1H), 2.39-2.34 (complex m, 2H), 2.27-2.20 (complex m, 2H), 1.70 (s, 3H), 1.62 (s, 3H), 1.62 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 156.6, 133.7, 121.58, 118.2 (q, *J* = 337 Hz), 104.3, 33.9, 25.6, 24.6, 17.6.

IR (KBr) ν_{max} 2972, 2920, 2861, 1670, 1419, 1248, 1210, 1145, 933, 899, 848, 705, 612 cm^{-1}.

trimethyl(7-methyl-3-methyleneoct-6-en-1-ynyl)silane (194)

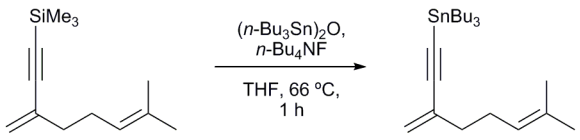


193 **246** **194**

A magnetically stirred solution of enol triflate **193** (6.82 g, 26.4 mmol) in THF (70 mL) and piperidine (30 mL) maintained at 20 °C under a nitrogen atmosphere was treated with cuprous iodide (200 mg, 1.05 mmol) and dichloro*bis*(acetonitrile)palladium (139 mg, 0.540 mmol). The ensuing yellow mixture was treated, over a period of 0.3 h, with trimethylsilylacetylene (**246**) (3.90 g, 39.7 mmol, *ex* Aldrich) then stirred at 20 °C for a further 0.25 h at which point the conspicuous formation of palladium black indicated that reaction was complete. Accordingly, the reaction mixture was quenched with HCl (150 mL of a 0.5 M aqueous solution) then extracted with hexane (2 × 100 mL). The combined organic phases were washed with $NaHCO_3$ (1 × 100 mL of a saturated aqueous solution) and brine (1× 100 mL) before being dried ($MgSO_4$), filtered and concentrated under reduced pressure. The resulting black oil was subjected to flash chromatography (silica, hexane elution) and concentration of the relevant fractions (R_f = 0.4) furnished dienyne **194**[101,102] (4.48 mg, 82%) as a pale-yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.36 (m, 1H), 5.23 (m, 1H), 5.23 (m, 1H), 5.12-5.08 (complex m, 1H), 2.20-2.16 (complex m, 4H), 1.69 (s, 3H), 1.62 (s, 3H), 0.19 (s, 9H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 132.2, 131.4, 123.3, 122.0, 105.6, 93.9, 37.3, 26.7, 25.7, 17.7, 0.0.
IR (KBr) ν_{max} 2963, 2144, 1606, 1448, 1376, 1250, 1105, 975, 942, 883, 842, 759, 698, 633 cm^{-1}.
Mass spectrum (EI, 70 eV) *m/*z 206 ($M^{+\bullet}$, 6%), 191 (16), 163 (99), 97 (64), 73 (100), 69 (75), 59 (23).
HREIMS Found $M^{+\bullet}$, 206.1490. $C_{13}H_{22}Si$ requires $M^{+\bullet}$, 206.1491.

tri-*n*-butyl(7-methyl-3-methyleneoct-6-en-1-ynyl)stannane (190)



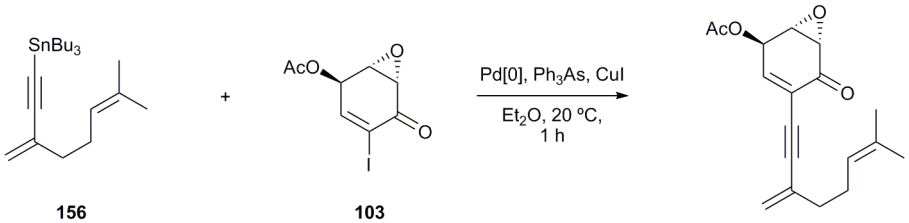
194 **190**

A magnetically stirred solution of compound **194** (2.07 mg, 10.0 mmol, *ex* Aldrich) and *bis*(tri-*n*-butyltin)oxide (2.99 g, 5.00 mmol) in THF (35 mL) maintained under a nitrogen atmosphere was treated with tetra-*n*-butylammonium fluoride (0.50 mL of a 1 M solution in THF,

0.50 mmol). The ensuing mixture was heated at reflux for 3 h then concentrated under reduced pressure to yield a biphasic oil that consisted of a pale-yellow layer and a denser, dark-brown layer. Subjection of this mixture to flash chromatography (silica, hexane elution) and concentration of the appropriate fractions (R_f = 0.4) gave the title compound **190**[101,102] (860 mg, 20%) as a clear, colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.31 (m, 1H), 5.16 (m, 1H), 5.11 (m, 1H), 2.23-2.15 (m, 4H), 1.69 (s, 3H), 1.62 (s, 3H), 1.63-1.51 (complex m, 6H), 1.37-1.30 (complex m, 6H), 1.02-0.97 (complex m, 6H), 0.90 (t, *J* = 7.5 Hz, 9H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 132.1, 132.0, 123.5, 120.7, 110.5, 92.8, 37.7, 28.9, 27.0, 26.8, 25.7, 17.7, 13.7, 11.1.
IR (KBr) ν_{max} 3313, 2958, 2920, 2124, 1609, 1455, 1376, 1270, 1073, 960, 895, 696, 671, 602, 513 cm^{-1}.

Compound 173 [(−)-tricholomenyn A] (*via* Route A)



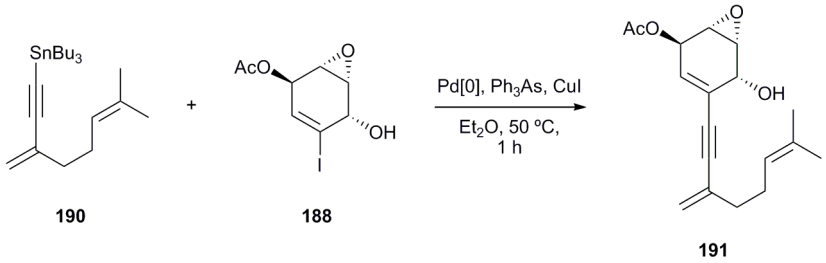
156 **103** **173**

A magnetically stirred solution of compound **103** (138 mg, 0.470 mmol) and compound **156** (300 mg, 0.710 mmol) in diethyl ether (2 mL) was treated with palladium(II) acetate (10 mg, 0.045 mmol), copper(I) iodide (10 mg, 0.053 mmol) and triphenylarsine (43 mg, 0.14 mmol). The ensuing mixture was stirred at 50 °C for 1 h, cooled and then concentrated under reduced pressure to afford a yellow oil. Subjection of this material to flash chromatography (silica, 1:4 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.25) then gave the title compound **173**[90-92,101,102,130] (102 mg, 72%) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.75 (ddd, *J* = 5.4 and 2.4 Hz, 1H), 5.81 (dt, *J* = 5.4 and 1.2 Hz, 1H), 5.44 (m, 1H), 5.35 (broad s, 1H), 5.08 (m, 1H), 3.74 (m, 1H), 3.59 (dd, *J* = 3.6 and 1.2 Hz, 1H), 2.20 (broad s, 4H), 2.14 (s, 3H), 1.68 (s, 3H), 1.62 (s, 3H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 189.5, 169.6, 140.3, 132.4, 130.5, 125.0, 123.5, 123.0, 95.9, 81.9, 64.1, 54.7, 52.9, 36.9, 26.6, 25.7, 20.6, 17.7.
IR (KBr) ν_{max} 2925, 2205, 1758, 1741, 1702, 1617, 1437, 1372, 1220, 1025, 987, 908 cm^{-1}.
Mass spectrum (EI, 70 eV) *m/z* 300 ($M^{+\bullet}$, 1%), 257 (27), 241 (39), 211 (41), 115 (36), 69 (93), 43 (100), 41 (85).
HREIMS Found: $M^{+\bullet}$, 300.1358. $C_{18}H_{20}O_4$ requires $M^{+\bullet}$, 300.1362.

Optical rotation $[\alpha]_D$ −241.3 (*c* 1.35, CH_2Cl_2) {lit.[130] $[\alpha]_D$ −148.1 (*c* 0.35, CH_2Cl_2); lit.[90] $[\alpha]_D$ −237 (*c* 0.6, CH_2Cl_2); lit.[92] $[\alpha]_D$ −228 (*c* 0.1, CH_2Cl_2)}.

(1*S*,2*R*,5*R*,6*R*)-5-hydroxy-4-(7-methyl-3-methyleneoct-6-en-1-ynyl)-7-oxabicyclo[4.1.0]hept-3-en-2-yl acetate (191)



190 **188** **191**

A magnetically stirred solution of compound **188** (256 mg, 0.860 mmol) and compound **190** (550 mg, 1.30 mmol) in diethyl ether (2.5 mL) was treated with palladium(II) acetate (20 mg, 0.089 mmol), copper(I) iodide (16 mg, 0.084 mmol) and triphenylarsine (80 mg, 0.26 mmol). The ensuing mixture was stirred at 50 °C for 1 h then cooled and concentrated under reduced pressure to afford a brown oil. Subjection of this material to flash chromatography (silica, 1:3, v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.3) then gave the *title compound **191*** (77 mg, 29%) as a pale-yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.96 (m, 1H), 5.55 (broad d, *J* = 5.4 Hz, 1H), 5.38 (m, 1h), 5.29 (m, 1H), 5.08 (m, 1H), 4.51 (broad m, 1H), 3.61 (m, 1H), 3.43 (m, 1H), 2.44 (d, *J* = 8.4 Hz, 1H), 2.19 (m, 4H), 2.08 (s, 3H), 1.68 (s, 3H), 1.60 (s, 3H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 169.9, 132.4, 130.6, 127.3, 125.4, 123.0, 122.7, 93.2, 85.6, 65.2, 64.6, 53.3, 52.8, 37.1, 26.7, 25.7, 20.8, 17.7.
IR (KBr) ν_{max} 3458, 2926, 1739, 1632, 1443, 1371, 1230, 1059, 1021, 896 cm^{-1}.
Mass spectrum (ESI, 70 eV) *m/*z 345 (100), 325 [$(M+Na)^+$, 81], 283 (5), 265 (14).
HREIMS Found: $(M+Na)^+$, 325.1418. $C_{18}H_{22}O_4$ requires $(M+Na)^+$, 325.1416.
Optical rotation $[\alpha]_D$ −85.7 (*c* 3.6, $CHCl_3$).

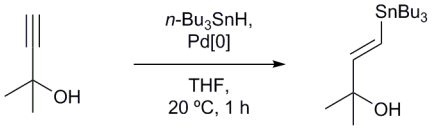
Compound 174 [(−)-tricholomenyn A] (*via* Route B)

191 **174**

A magnetically stirred solution of compound **191** (36 mg, 0.12 mmol) in DCM (2.5 mL) was treated with acetic acid (10 µL, 0.18 mmol) and pyridinium dichromate (61 mg, 0.16 mmol). The ensuing mixture was stirred at 20 °C for 2 h and then treated with silica gel (2 mL) and diluted with diethyl ether (2.5 mL). The mixture was filtered and the filtrate was concentrated under reduced pressure to a pale-orange oil. Subjection of this material to flash chromatography (silica, 1:4 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.2) then gave the *title compound* ***174***[90-92,101,102,130] (24 mg, 67%) as a pale-yellow oil. The spectral data derived from this material matched those obtained from the sample of compound **174** generated directly *via* cross-coupling of stannane **156** and iodide **103** as detailed above.

4.4 EXPERIMENTAL PROCEDURES FOR CHAPTER THREE

(*E*)-2-methyl-4-(tri-*n*-butylstannyl)but-3-en-2-ol (229)



232 **229**

A magnetically stirred solution of propargyl alcohol **232** (3.01 g, 35.8 mmol, *ex* Aldrich) in THF (100 mL) was treated with dichlorobis(triphenylphosphine)palladium(II) (500 mg, 0.712 mmol) then, dropwise, with tri-*n*-butyltin hydride (9.5 mL, 35.8 mmol). The ensuing mixture was maintained at 20 °C for 1 h and then concentrated under reduced pressure to afford a black oil. This material was treated with hexane and the resulting mixture passed through a pad of TLC-grade silica gel. Concentration of the filtrate afforded the title compound **229**[136] (12.6 g, 94%) as a clear, colorless oil.

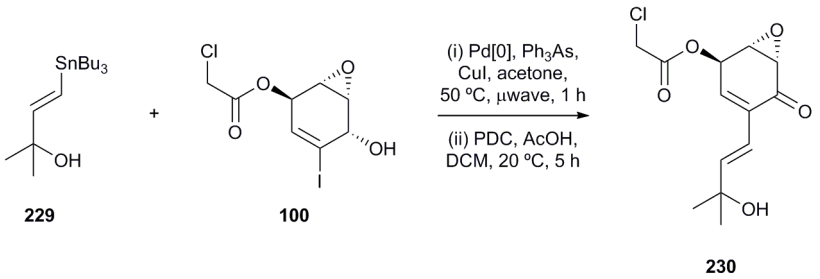
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.10 (m, 2H), 1.60-1.20 (complex m, 18H), 1.29 (s, 6H), 0.88 (t, *J* = 7.2 Hz, 9H) (signal due to hydroxyl group proton not observed).
^{13}C NMR (75 MHz, $CDCl_3$) δ 155.5, 122.3[dt, $^1J(^{13}C\text{-}^{119}Sn)$ = 752.6 Hz and $^1J(^{13}C\text{-}^{117}Sn)$ = 719.4 Hz], 72.4, 29.4, 29.0 [t, $^3J(^{13}C\text{-}^{117/119}Sn)$ = 40.8 Hz], 27.2 [t, $^2J(^{13}C\text{-}^{117/119}Sn)$ = 108.6 Hz], 13.7, 9.4 [dt, $^1J(^{13}C\text{-}^{119}Sn)$ = 682.8 Hz and $^1J(^{13}C\text{-}^{117}Sn)$ = 652.8 Hz].

IR (KBr) ν_{max} 3364, 2957, 2925, 2871, 2854, 1600, 1463, 1376, 989, 666 cm^{-1}.

Mass spectrum (ESI, 70 eV) *m/z* 375 [$(M-H^{\bullet})^+$, 5%], 315 (100), 263 (60), 207 (50), 177 (45), 137 (40), 59 (40), 41 (40).

HRESIMS Found: $(M-H^{\bullet})^+$, 375.1718. $C_{17}H_{36}O_4{}^{120}Sn$ requires $(M-H^{\bullet})^+$, 375.1710.

(1*S*,2*R*,6*S*)-4-((*E*)-3-hydroxy-3-methylbut-1-enyl)-5-oxo-7-oxabicyclo[4.1.0]hept-3-en-2-yl 2-chloroacetate (230)



A magnetically stirred solution of iodide **100** (398 mg, 1.20 mmol) and stannane **229** (1.37 g, 3.64 mmol) in acetone (5 mL) was treated with palladium(II) acetate (24 mg, 0.11 mmol), copper(I) iodide (20 mg, 0.11 mmol) and triphenylarsine (96 mg, 0.32 mmol). The ensuing mixture was stirred in a sealed tube at 50 °C with microwave heating for 0.3 h then cooled and concentrated under reduced pressure to afford a dark-brown oil. Subjection of this material to flash chromatography (silica, 1:1 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.3) then gave the *alcoholic precursor to compound **230*** (165 mg, 47%) as a clear, colorless oil. A magnetically stirred solution of this alcohol (156 mg, 0.54 mmol) in DCM (5 mL) was treated with acetic acid (45 μL, 0.79 mmol) and pyridinium dichromate (263 mg, 0.70 mmol). The enxuing mixture was stirred at 20 °C for 1 h and then diluted with diethyl ether (5 mL) and the ensuing mixture passed through a pad of TLC-grade silica gel. The filtrate was then concentrated under reduced pressure to give a dark-brown oil. Subjection of this material to flash chromatography (silica, 2:5 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.3) gave the *title compound **230*** (106 mg, 68%) as a clear, colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.48 (dd, *J* = 5.4 and 2.4 Hz, 1H), 6.44 (d, *J* = 16.2 Hz, 1H), 6.29 (d, *J* = 16.2 Hz, 1H), 5.86 (dt, *J* = 5.4 and 1.2 Hz, 1H), 4.10 (ABq, *J* = 14.0 Hz, 2H), 3.77 (m, 1H), 3.59 (dd, *J* = 3.6 and 1.2 Hz, 1H), 1.33 (s, 3H), 1.31 (s, 3H) (signal due to hydroxyl group proton not observed).

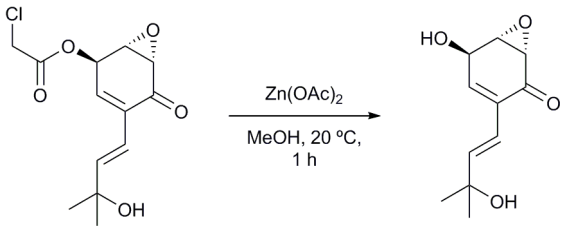
^{13}C NMR (75 MHz, $CDCl_3$) δ 192.7, 166.5, 144.0, 136.3, 131.0, 118.9, 71.0, 66.5, 54.1, 63.5, 40.4, 29.5(1), 29.4(9).

IR (KBr) ν_{max} 3447, 2971, 1764, 1689, 1603, 1251, 1166, 984 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/z* 271 ([$M - CH_3^{\bullet}$]$^+$, 5%), 177 (15), 150 (55), 134 (60), 43 (100).

HREIMS Found: $(M-CH_3^{\bullet})^+$, 271.0381. $C_{13}H_{15}{}^{35}ClO_5$ requires $(M-CH_3^{\bullet})^+$, 271.0373.
Optical rotation $[\alpha]_D$ −101.5 (*c* 1.55, Et_2O).

(1*S*,5*R*,6*S*)-5-hydroxy-3-((*E*)-3-hydroxy-3-methylbut-1-enyl)-7-oxabicyclo[4.1.0]hept-3-en-2-one (199)

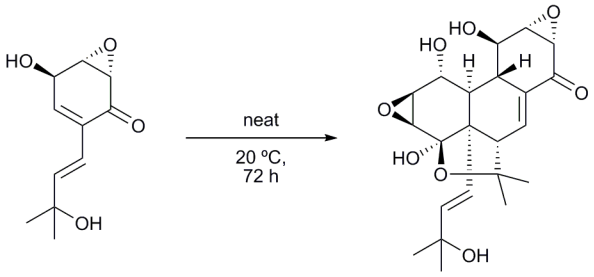


230 **199**

A magnetically stirred solution of compound **230** (40 mg, 0.14 mmol) in methanol (4 mL) was treated with zinc(II) acetate dihydrate (15 mg, 0.07 mmol). The ensuing mixture was stirred at 20 °C for 1 h and then concentrated under reduced pressure to afford a white solid. This material was treated with ethyl acetate and the ensuing mixture passed through a pad of TLC-grade silica gel. Concentration of the filtrate under reduced pressure then gave a pale-yellow oil that was subjected to flash chromatography (silica, 1:1 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.3) afforded the title compound **199**[95] (24 mg, 82%) as a clear oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.57 (dd, *J* = 5.1 and 2.7 Hz, 1H), 6.35 (d, *J* = 16.2 Hz, 1H), 6.22 (d, *J* = 16.2 Hz, 1H), 4.71 (broad d, *J* = 5.1 Hz, 1H), 3.80 (m, 1H), 3.53 (dd, *J* = 3.9 and 1.2 Hz, 1H), 1.34 (s, 3H), 1.32 (s, 3H) (signal due to the hydroxyl groups protons not observed).
^{13}C NMR (75 MHz, $CDCl_3$) δ 194.5, 143.0, 138.1, 133.8, 119.7, 71.5, 63.6, 57.8, 54.5, 29.7(4), 29.7(0).
IR (KBr) ν_{max} 3411, 2925, 1683, 1378, 1259, 1149, 1019 cm^{-1}.
Mass spectrum (EI, 70 eV) *m/z* 195 ($[M - CH_3^{\bullet}]^+$, 10%), 149 (20), 134 (20), 134 (45), 43 (100).
HREIMS Found: $(M - CH_3^{\bullet})^+$, 195.0656. $C_{11}H_{14}O_4$ requires $(M - CH_3^{\bullet})^+$, 195.0657.
Optical rotation $[\alpha]_D$ −155.0 (c 1.3, acetone).

Compound 192 [(+)-panepophenanthrin]



199 **192**

A neat sample of compound **199** (25 mg, 0.12 mmol) was stored at 20 °C for 72 h and thereby afforded the title compound **192**[95,99,134] (25 mg, quantitative) as a white, crystalline solid, m.p. = 140-144 °C [lit.[99] m.p. = 145-148 °C].

^{1}H NMR (300 MHz, CD_3OD) δ 6.77 (dd, *J* = 4.5 and 2.7 Hz, 1H), 5.96 (d, *J* = 16.5 Hz, 1H), 5.64 (d, *J* = 16.5 Hz, 1H), 4.52 (broad s, 1H), 4.32 (broad s, 1H), 3.80 (t, *J* = 3.6 Hz, 1H), 3.46 (t, *J* = 3.6 Hz, 1H), 3.39 (d, *J* = 4.2 Hz, 1H), 3.28 – 3.26 (m, 2H), 2.28 (d, *J* = 9.3 Hz, 1H), 1.99 (d, *J* = 9.3 Hz, 1H), 1.41 (s, 3H), 1.31 (s, 3H), 1.16 (s, 3H), 1.13 (s, 3H) (signal due to hydroxyl group protons not observed).

^{13}C NMR (75 MHz, CD_3OD) δ 196.2, 142.9, 10.0, 138.8, 129.3, 102.7, 79.1, 71.8, 69.0, 66.2, 60.6, 57.4, 57.2, 57.1, 55.6, 55.1, 51.2, 49.9, 32.3, 30.3, 29.5, 26.2.

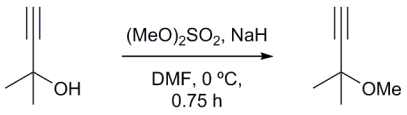
IR (KBr) ν_{max} 3422, 2972, 1685, 1599, 1369, 1255, 1148, 1016 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/*z 459 ($[M+K]^+$, 25%), 443 [$(M+Na)^+$, 50], 332 (100), 330 (75), 328 (43).

HREIMS Found: $(M+Na)^+$, 443.1672. $C_{22}H_{28}O_8$ requires $(M+Na)^+$, 443.1682.

Optical rotation $[\alpha]_D$ +144.0 (*c* 0.5, methanol) {lit.[99] $[\alpha]_D$ +146 (*c* 1.0, methanol)}.

3-methoxy-3-methylbut-1-yne (243)

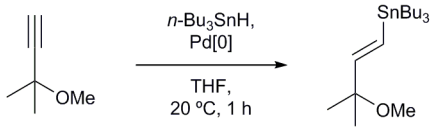


232 **243**

A magnetically stirred solution of compound **232** (8.40 g, 0.100 mmol) in DMF (100 mL) was treated with sodium hydride (6.09 g of 60% w/w dispersion in mineral oil, 0.152 mmol) and the resulting mixture stirred at 0 °C for 0.5 h. Dimethyl sulfate (14.5 mL, 0.152 mmol) was then added dropwise and then after the addition was complete the reaction mixture was warmed to 20 °C over 0.75 h. After this time acetic acid (5 mL) was added to the reaction mixture which was then distilled to give the title compound **243**[142,146] (8.67 g, 88%) as a clear oil, b.p. = 78-80 °C @ 760 mm Hg (lit.[142] b.p. = 78-80 °C @ 760 mm Hg).

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.34 (s, 3H), 2.41 (s, 1H), 1.43 (s, 6H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 85.5, 72.0, 70.2, 51.5, 28.1.

(*E*)-tri-*n*-butyl(3-methoxy-3-methylbut-1-enyl)stannane (241)

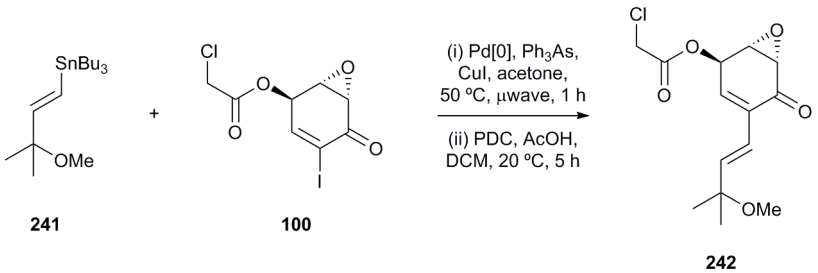


243 **241**

A magnetically stirred solution of compound **243** (2.00g, 20.4 mmol) in THF (80 mL) was treated with dichlorobis(triphenylphosphine)palladium(II) (145 mg, 0.21 mmol) and, dropwise, with tri-*n*-butyltin hydride (5.1 mL, 19.0 mmol). The ensuing mixture was maintained at 20 °C for 1 h and then concentrated under reduced pressure to afford a black oil. This material was diluted with hexane (50 mL) and passed through a pad of TLC-grade silica gel. Concentration of the filtrate under reduced pressure then gave the title compound **241**[93] (6.10 g, 83%) as a pale-yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.05 (d, *J* = 19.2 Hz, 1H), 5.89 (d, *J* = 19.2 Hz, 1H), 3.14 (s, 3H), 1.48 (m, 6H), 1.30 (m, 6H), 1.25 (s, 6H), 0.88 (m, 15H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 153.0, 126.7, 76.6, 50.4, 29.1, 27.2, 25.3, 13.7, 9.4.

(1*S*,2*R*,6*S*)-4-((*E*)-3-methoxy-3-methylbut-1-enyl)-5-oxo-7-oxabicyclo[4.1.0]hept-3-en-2-yl 2-chloroacetate (242)



241 **100** **242**

A magnetically stirred solution of iodide **100** (385 mg, 1.16 mmol) and stannane **241** (1.37 g, 3.51 mmol) in acetone (5 mL) was treated with palladium(II) acetate (24 mg, 0.11 mmol), copper(I) iodide (20 mg, 0.11 mmol) and triphenylarsine (96 mg, 0.32 mmol). The ensuing mixture was stirred in a sealed tube at 50 °C with microwave heating for 0.3 h, then cooled and concentrated under reduced pressure to give a dark-brown oil. Subjection of this material to flash chromatography (silica, 1:3 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f = 0.3) then gave the *alcoholic precursor to compound **242*** (184 mg, 52%) as a pale-orange oil. A magnetically stirred solution of this alcohol (184 mg, 0.61 mmol)

in DCM (10 mL) was treated with acetic acid (45 μL, 0.79 mmol) and pyridinium dichromate (257 mg, 0.68 mmol). The ensuing mixture was stirred at 20 °C for 1 h and then diluted with diethyl ether before being passed through a pad of TLC-grade silica gel. The resulting filtrate was concentrated under reduced pressure to a dark-brown oil that was subjected to flash chromatography (silica, 1:4 v/v ethyl acetate/hexane elution). Concentration of the appropriate fractions (R_f = 0.3) then gave the *title compound* ***242*** (167 mg, 91%) as a clear, colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.48 (dd, *J* = 5.1 and 2.7 Hz, 1H), 6.26 (d, *J* = 16.2 Hz, 1H), 6.18 (d, *J* = 16.2 Hz, 1H), 5.85 (dt, *J* = 5.1 and 1.2 Hz, 1H), 4.10 (m, 2H), 3.75 (m, 1H), 3.57 (dd, *J* = 3.6 and 1.2 Hz, 1H), 1.33 (s, 3H), 1.31 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 192.4, 166.4, 141.7, 136.1, 130.8, 121.2, 74.9, 66.5, 54.1, 53.4, 50.5, 40.4, 25.6, 25.3.

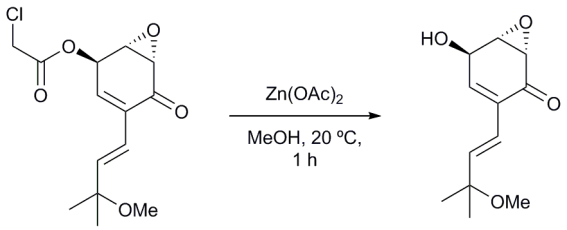
IR (KBr) ν_{max} 2977, 1766, 1692, 1250, 1167, 1071, 985 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/*z 300 ($M^{+\bullet}$, <1%), 287 and 285 (1 and 40), 207 (20), 191 (35), 175 (45), 163 (32), 147 (40), 77 (55), 73 (100).

HREIMS Found: $M^{+\bullet}$, 300.0764. $C_{14}H_{17}{}^{35}ClO_5$ requires $M^{+\bullet}$, 300.0765.

Optical rotation $[\alpha]_D$ −247.5 (*c* 3.6, $CHCl_3$).

(1*S*,5*R*,6*S*)-5-hydroxy-3-((*E*)-3-methoxy-3-methylbut-1-enyl)-7-oxabicyclo[4.1.0]hept-3-en-2-one (240)



242 **240**

A magnetically stirred solution of compound **242** (70 mg, 0.23 mmol) in methanol (4 mL) was treated with zinc(II) acetate dihydrate (7 mg, 0.3 μmol). The ensuing mixture was stirred at 20 °C for 1 h and then concentrated under reduced pressure to afford a white solid. This material was treated with ethyl acetate (5 mL) and passed through a pad of TLC-grade silica gel. The filtrate was concentrated under reduced pressure afforded a pale-yellow oil that was subjected to flash chromatography (silica, 1:2 v/v ethyl acetate/hexane elution). Concentration of the appropriate fractions (R_f = 0.3) then gave the title compound **240**[94] (41 mg, 78%) as a clear, colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.60 (dd, *J* = 5.4 and 3.0 Hz, 1H), 6.19 (s, 2H), 4.72 (ddd, *J* = 5.4 Hz, 1H), 3.78 (m, 1H), 3.53 (dd, *J* = 3.6 and 1.2 Hz, 1H), 3.12 (s, 3H), 1.27 (s, 6H) (signal due to the hydroxyl group proton not observed).

^{13}C NMR (75 MHz, $CDCl_3$) δ 193.6, 140.2, 137.4, 133.5, 121.8, 75.3, 63.3, 57.5, 54.1, 50.5, 25.6, 25.4.

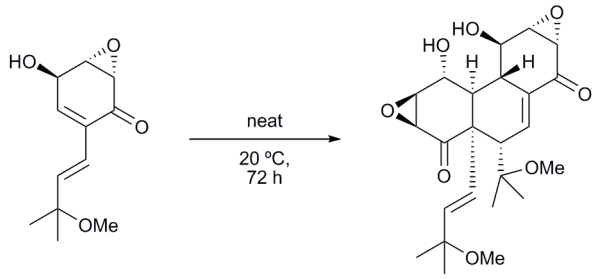
IR (KBr) ν_{max} 3385, 2976, 1687, 1380, 1250, 1170, 1058, 844 cm^{-1}.

Mass spectrum (EI, 70 eV) *m/*z 224 ($M^{+\bullet}$, <1%), 209 (30), 193 (15), 149 (20), 135 (20), 85 (80), 83 (100).

HREIMS Found: $M^{+\bullet}$, 224.1049. $C_{12}H_{16}O_4$ requires $M^{+\bullet}$, 224.1049.

Optical rotation $[\alpha]_D$ −139.9 (*c* 2.05, $CHCl_3$).

Compound 236 [(+)-Prehexacyclinol]



240 **236**

A neat sample of compound **240** (121 mg, 0.54 mmol) was stored at 20 °C for 72 h and so afforded the title compound **236**[93,94] (121 mg, quantitative) as a clear, colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.69 (m, 1H), 5.84 (d, *J* = 16.8 Hz, 1H), 5.40 (d, *J* = 16.8 Hz, 1H), 4.44 (broad s, 1H), 4.21 (m, 1H), 4.07 (d, *J* = 5.4 Hz, 1H), 3.72 (d, *J* = 5.4 Hz, 1H), 3.60 (m, 2H), 3.49 (d, *J* = 3.6 Hz, 1H), 3.44 (broad s, 1H), 3.35 (d, *J* = 3.6 Hz, 1H), 3.16 (s, 3H), 3.05 (s, 3H), 2.67 (m, 1H), 2.57 (s, 1H), 2.52 (broad s, 1H), 1.22 (s, 6H), 1.19 (s, 3H), 1.12 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 203.4, 194.9, 138.5, 137.9, 134.5, 130.8, 77.1, 75.5, 68.4, 573.5, 61.3, 59.4, 55.5, 53.6, 53.5, 50.6, 50.3, 48.4, 46.3, 26.0, 25.1, 24.4, 23.1 (signal due to one carbon obscured or overlapping).

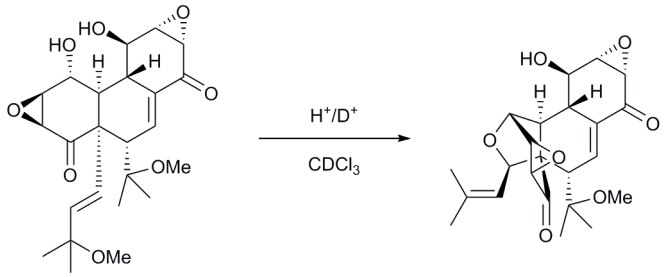
IR (KBr) ν_{max} 3424, 2974, 2926, 1699, 1626, 1248, 1059, 842 cm^{-1}.

Mass spectrum (ESI, 70 eV) *m/*z 919 [$(2M + Na)^+$, 100%], 471 [$(M + Na)^+$, 78], 318 (60).

HRESIMS Found: $(M + Na)^+$, 471.1997. $C_{24}H_{32}O_8$ requires $(M + Na)^+$, 471.1995.

Optical rotation $[\alpha]_D$ +30.8 (*c* 2.35, MeOH) {lit.[93] $[\alpha]_D$ +29.8 (*c* 0.40, CH_2Cl_2)}.

Compound 233 [(+)-Hexacyclinol]



236 **233**

A solution of compound **236** (38 mg, 0.085 mmol) in deuterochloroform (1 mL) was stored at 20 °C for 72 h and then concentrated under reduced pressure to afford the title compound **233**[93,94] (35 mg, 99%) as a white, crystalline solid, m.p. = 176-178 °C (lit.[94] m.p. = 179-180 °C).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.72 (dd, *J* = 6.0 and 2.4 Hz, 1H), 5.47 (d, *J* = 9.3 Hz, 1H), 5.00 (m, 1H), 4.82 (d, *J* = 9.3 Hz, 1H), 3.70-3.54 (complex m, 3H), 3.52 (d, *J* = 3.6 Hz, 1H), 3.29 (d, *J* = 2.7 Hz, 1H), 3.24 (d, *J* = 3.6 Hz, 1H), 3.02 (s, 3H), 2.74 (dd, *J* = 9.9 and 5.1 Hz, 1H), 1.77 (s, 3H), 1.72 (s, 3H), 1.27 (s, 3H), 1.15 (s, 3H) (signal due to hydroxyl group protons not observed).

^{13}C NMR (75 MHz, $CDCl_3$) δ 203.1, 193.2, 142.3, 139.6, 132.6, 120.5, 77.3, 75.7, 72.5, 71.4, 61.0, 60.3, 54.4, 53.2, 53.0, 49.1, 47.6, 40.6, 40.1, 26.6, 26.2, 24.6, 18.4.

IR (KBr) ν_{max} 3433, 2978, 1711, 1698, 1626, 1448, 1070, 752 cm^{-1}.

Mass spectrum (ESI, 70 eV) *m/*z 439 [$(M+Na)^+$, 100], 417 [$(M+H)^+$, 50], 323 (50), 193 (70), 99 (50), 73 (45).

HRESIMS Found: $(M + Na)^+$, 439.1724. $C_{23}H_{28}O_7$ requires $(M+Na)^+$, 439.1733.

Optical rotation $[\alpha]_D$ +129.6 (*c* 0.36, MeOH) {lit.[94] $[\alpha]_D$ +130.9 (*c* 0.42, MeOH)}.

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A.1 APPENDIX ONE

Comparative NMR data recorded for naturally- and synthetically derived tambjamines

Table 5-1: *Comparison of the 1H NMR data recorded on naturally- and synthetically-derived tambjamine C (**4**) , isolated as the free base and acetic acid salt, respectively.*

^{1}H NMR Data Natural **4** (δ_H) (200 MHz, $CDCl_3$)[6]	^{1}H NMR Data Synthetic **4** (δ_H) (300 MHz, $CDCl_3$)
10.7 (broad s, 1H)	11.1 (broad s, 2H)
9.46 (broad s, 1H)	
7.29 (d, *J* = 15 Hz, 1H)	7.28 (s, 1H)
7.07 (m, 1H)	7.04 (m, 1H)
6.73 (m, 1H)	6.70 (m, 1H)
6.28 (m, 1H)	6.24 (m, 1H)
5.94 (s, 1H)	5.95 (s, 1H)
3.93 (s, 3H)	3.90 (s, 3H)
3.29 (m, 2H)	3.24 (d, *J* = 6.6 Hz, 1H)
	2.08 (s, 3H, $CH_3CO_2^-$)
2.03 (m, 1H)	1.98 (m, 1H)
1.03 (d, *J* = 6.6 Hz, 6H)	0.99 (d, *J* = 6.6 Hz, 6H)

Table 5-2: *Comparison of the ^{13}C and ^{1}H NMR data recorded on naturally- and synthetically-derived tambjamine E (**2**), isolated as the free base and acetic acid salt, respectively.*

^{13}C NMR Data Natural **2** (δ_C) (50 MHz, $CDCl_3$)[145]	^{13}C NMR Data Synthetic **2** (δ_C) (75 MHz, $CDCl_3$)	^{1}H NMR Data Natural **2** (δ_H) (360 MHz, $CDCl_3$)[145]	^{1}H NMR Data Synthetic **2** (δ_H) (300 MHz, $CDCl_3$)
	179.2	9.92 (broad s, 1H)	11.1 (broad s, 2H)
164.6	162.9	9.50 (broad s, 1H)	
142.8	143.6	7.36 (broad m, 1H)	7.30 (s, 1H)
142.2	140.5	7.07 (broad m, 1H)	6.83 (m, 1H)
123.8	124.9	6.76 (broad m, 1H)	6.57 (m, 1H)
123.7	121.8	6.28 (broad m, 1H)	6.19 (m, 1H)
113.0	114.2	5.97 (s, 1H)	5.94 (s, 1H)
111.4	110.4	3.93 (s, 3H)	3.85 (s, 3H)
111.2	109.6	3.56 (broad q, *J* = 7.3 Hz, 2H)	3.32 (q, *J* = 7.5 Hz, 2H)
92.3	91.6		2.10 (s, 3H, $CH_3CO_2^-$)
59.1	68.0	1.41 (t, *J* = 7.3 Hz, 3H)	1.18 (t, *J* = 7.5 Hz, 3H)
45.8	45.6		
	24.7		
15.5	15.7		

Table 5-3: *Comparison of the ^{13}C and ^{1}H NMR data recorded on naturally- and synthetically-derived tambjamine F (**3**), isolated as the free base and acetic acid salt, respectively.*

^{13}C NMR Data Natural **3** (δ_C) (50 MHz, $CDCl_3$)[145]	^{13}C NMR Data Synthetic **3** (δ_C) (75 MHz, $CDCl_3$)	^{1}H NMR Data Natural **3** (δ_H) (360 MHz, $CDCl_3$)[145]	^{1}H NMR Data Synthetic **3** (δ_H) (300 MHz, $CDCl_3$)
	179.1	9.88 (broad s, 1H)	12.2 (s, 2H)
164.7	163.5	7.35-7.20 (complex m, 6H)	7.32-7.19 (complex m, 5H)
143.0	142.8	6.70 (broad m, 1H)	7.08 (s, 1H), 6.68 (m, 1H)
142.3	140.5	6.27 (broad m, 1H)	6.25 (m, 1H)
138.7	137.4	5.93 (s, 1H)	5.89 (s, 1H)
129.8	128.8	3.87 (s, 3H)	3.81 (s, 3H)
129.3	128.6	3.68 (t, *J* = 7.2 Hz, 2H)	3.64 (t, *J* = 7.2 Hz, 2H)
127.3	126.6	3.03 (t, *J* = 7.2 Hz, 2H)	3.00 (t, *J* = 7.2 Hz, 2H)
123.9	123.5		2.10 (s, 3H, $CH_3CO_2^-$)
123.6	122.9		
113.2	112.5		
113.2	110.8		
113.2	110.0		
92.2	91.1		
59.0	58.0		
52.5	52.3		
36.7	36.6		
	24.6		

Table 5-4: *Comparison of the ^{13}C and ^{1}H NMR data recorded on naturally- and synthetically-derived BE-18591 (**5**), isolated as the free base and acetic acid salt, respectively.*

^{13}C NMR Data Natural **5** (δ_C) (75 MHz, $CDCl_3$)[35]	^{13}C NMR Data Synthetic **5** (δ_C) (75 MHz, $CDCl_3$)	^{1}H NMR Data Natural **5** (δ_H) (300 MHz, $CDCl_3$)[35]	^{1}H NMR Data Synthetic **5** (δ_H) (300 MHz, $CDCl_3$)
	179.0	10.8 (broad s, 1H)	11.4 (s, 2H)
163.6	163.2	9.50 (broad s, 1H)	
142.4	142.6	7.33 (broad d, *J* = 9.0 Hz, 1H)	7.26 (s, 1H)
140.0	140.5	7.06 (m, 1H)	7.00 (m, 1H)
124.1	123.4	6.74 (m, 1H)	6.64 (m, 1H)
122.3	123.1	6.28 (m, 1H)	6.21 (m, 1H)
113.2	112.3	5.96 (s, 1H)	5.89 (s, 1H)
110.6	110.9	3.93 (s, 3H)	3.83 (s, 3H)
110.6	109.9	3.47 (broad t, *J* = 7.0 Hz, 2H)	3.37 (t, *J* = 7.1 Hz, 2H)
91.9	91.0		2.06 (s, 3H, $CH_3CO_2^-$)
58.4	58.0	1.75 (m, 2H)	1.66 (m, 2H)
51.0	50.9	1.20-1.45 (m, 18H)	1.22 (m, 18H)
31.9	31.8	0.88 (t, *J* = 7.0 Hz, 3H)	0.85 (t, *J* = 6.8 Hz, 3H)
30.2	30.3		
29.6			
29.6	29.5		
29.6	29.4		
29.4	29.3		
29.3	29.2		
29.1	29.1		
26.5	26.4		
22.7	24.6		
14.1	22.6		
	14.0		

Table 5-5: *Comparison of the ^{13}C and ^{1}H NMR data recorded on naturally- and synthetically-derived tambjamine **6**, isolated as the free base and acetic acid salt, respectively.*

^{13}C NMR Data Natural **6** (δ_C) (75 MHz, $CDCl_3$)[2]	^{13}C NMR Data Synthetic **6** (δ_C) (75 MHz, $CDCl_3$)	^{1}H NMR Data Natural **6** (δ_H) (300 MHz, $CDCl_3$)[2]	^{1}H NMR Data Synthetic **6** (δ_H) (300 MHz, $CDCl_3$)
	179.0		11.4 (s, 2H)
163.9	163.5	7.29 (d, *J* = 14 Hz, 1H)	7.28 (s, 1H)
143.4	142.9	7.06 (m, 1H)	7.04 (m, 1H)
141.1	140.6	6.70 (m, 1H)	6.69 (m, 1H)
134.5	134.1	6.25 (m, 1H)	6.25 (m, 1H)
124.1	123.9	5.95 (s, 1H)	5.94 (s, 1H)
124.0	123.7	5.55 (dt, *J* = 10.8 Hz, 1H)	5.54 (m, 1H)
123.5	123.1	5.35 (dt, *J* = 10.8 Hz, 1H)	5.34 (m, 1H)
113.0	112.5	3.90 (s, 3H)	3.89 (s, 3H)
111.3	110.9	3.46 (m, 2H)	3.45 (t, *J* = 6.9 Hz, 2H)
110.5	110.1	2.46 (m, 2H)	2.45 (m, 2H)
91.6	91.2		2.09 (s, 3H, $CH_3CO_2^-$)
58.8	58.2	2.01 (m, 2H)	2.02 (m, 2H)
51.2	50.8	1.70-1.21 (complex m, 12H)	1.22 (m, 12H)
28.8	31.8	0.86 (t, 3H)	0.85 (t, *J* = 6.8 Hz, 3H)
27.8	29.7		
27.7-26.5 (6C)	29.5		
	29.3		
	29.3		
	28.4		
	27.4		
	24.5		
	22.6		
14.5	14.1		

Table 5-6: *Comparison of the ^{13}C and ^{1}H NMR data recorded on naturally- and synthetically-derived tambjamine G (**8**), both isolated as their acetic acid salt*

^{13}C NMR Data Natural **8** (δ_C) (75 MHz, $CDCl_3$)[42]	^{13}C NMR Data Synthetic **8** (δ_C) (75 MHz, $CDCl_3$)	^{1}H NMR Data Natural **8** (δ_H) (300 MHz, $CDCl_3$)[42]	^{1}H NMR Data Synthetic **8** (δ_H) (300 MHz, $CDCl_3$)
179.0	179.7	12.85 (broad s, 1H)	11.10 (broad s, 2H)
164.0	163.6	11.5 (broad s, 1H)	
142.0	141.6	7.30 (broad s, 1H)	7.34 (s, 1H)
141.4	140.9	6.57 (d, *J* = 4.2 Hz, 1H)	6.58 (d, *J* = 3.9 Hz, 1H)
125.1	124.6	6.18 (d, *J* = 4.2 Hz, 1H)	6.16 (d, *J* = 3.9 Hz, 1H)
114.0	113.8	5.87 (s, 1H)	5.89 (s, 1H)
112.8	112.5	3.68 (s, 3H)	3.87 (s, 3H)
111.6	111.2	3.49 (q, *J* = 8.0 Hz, 2H)	3.50 (t, *J* = 7.2 Hz, 2H)
105.6	105.4	2.10 (s, 3H, $CH_3CO_2^-$)	2.11 (s, 3H, $CH_3CO_2^-$)
91.4	91.1	1.36 (t, *J* = 8.0 Hz, 3H)	1.36 (t, *J* = 7.2 Hz, 3H)
58.9	58.5		
46.3	45.9		
24.1	24.9		
16.2	15.8		

Table 5-7: *Comparison of the ^{13}C and ^{1}H NMR data recorded on naturally- and synthetically-derived tambjamine H (**9**), both isolated as their acetic acid salt*

^{13}C NMR Data Natural **9** (δ_C) (75 MHz, $CDCl_3$)[42]	^{13}C NMR Data Synthetic **9** (δ_C) (75 MHz, $CDCl_3$)	^{1}H NMR Data Natural **9** (δ_H) (300 MHz, $CDCl_3$)[42]	^{1}H NMR Data Synthetic **9** (δ_H) (300 MHz, $CDCl_3$)
179.0	179.1	12.9 (broad s, 1H)	11.30 (broad s, 2H)
164.1	163.3	11.5 (broad s, 1H)	
142.2	141.3	7.39 (broad s, 1H)	7.29 (s, 1H)
141.8	141.1	6.63 (d, *J* = 4.2 Hz, 1H)	6.53 (d, *J* = 3.6 Hz, 1H)
125.1	124.4	6.23 (d, *J* = 4.2 Hz, 1H)	6.14 (d, *J* = 3.6 Hz, 1H)
114.1	113.3	5.94 (s, 1H)	5.84 (s, 1H)
112.9	112.1	3.95 (s, 3H)	3.84 (s, 3H)
111.6	110.8	3.46 (t, *J* = 8.0 Hz, 2H)	3.37 (t, *J* = 7.1 Hz, 2H)
105.9	104.9	2.10 (s, 3H, $CH_3CO_2^-$)	2.08 (s, 3H, $CH_3CO_2^-$)
91.5	91.0	1.78 (m, 2H)	1.71 (m, 2H)
58.9	58.1	1.04 (t, *J* = 8.0 Hz, 3H)	0.96 (t, *J* = 7.1 Hz, 3H)
53.5	52.6		
30.4	24.5		
24.1	23.6		
11.7	10.9		

Table 5-8: *Comparison of the ^{13}C and ^{1}H NMR data recorded on naturally- and synthetically-derived tambjamine I (**10**), both isolated as their acetic acid salt*

^{13}C NMR Data Natural **10** (δ_C) (75 MHz, $CDCl_3$)[42]	^{13}C NMR Data Synthetic **10** (δ_C) (75 MHz, $CDCl_3$)	^{1}H NMR Data Natural **10** (δ_H) (300 MHz, $CDCl_3$)[42]	^{1}H NMR Data Synthetic **10** (δ_H) (300 MHz, $CDCl_3$)
179.0	179.2	12.9 (broad s, 1H)	11.10 (broad s, 2H)
164.2	163.4	11.5 (broad s, 1H)	
142.2	141.5	7.29 (broad s, 1H)	7.26 (s, 1H)
142.2	141.3	6.59 (d, *J* = 4.2 Hz, 1H)	6.55 (d, *J* = 3.6 Hz, 1H)
125.1	124.4	6.19 (d, *J* = 4.2 Hz, 1H)	6.15 (d, *J* = 3.6 Hz, 1H)
114.3	113.5	5.89 (s, 1H)	5.86 (s, 1H)
113.0	112.1	3.89 (s, 3H)	3.86 (s, 3H)
111.6	110.8	3.28 (d, *J* = 7.5 Hz, 2H)	3.23 (d, *J* = 6.9 Hz, 2H)
106.0	105.2	2.10 (s, 3H, $CH_3CO_2^-$)	2.08 (s, 3H, $CH_3CO_2^-$)
91.5	90.7	1.99 (m, 1H)	1.97 (m, 1H)
59.3	58.6	1.00 (d, *J* = 7.5 Hz, 6H)	0.97 (d, *J* = 6.9 Hz, 6H)
58.9	58.2		
30.2	29.4		
24.1	24.5		
20.4 (2C)	19.7 (2C)		

Table 5-9: *Comparison of the ^{13}C and ^{1}H NMR data recorded on naturally- and synthetically-derived tambjamine J (**11**), both isolated as their acetic acid salt*

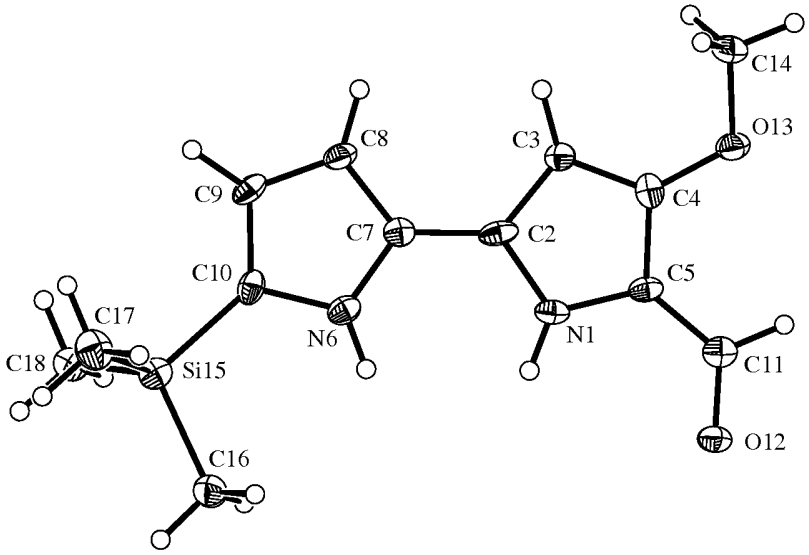
^{13}C NMR Data Natural **11** (δ_C) (75 MHz, $CDCl_3$)[42]	^{13}C NMR Data Synthetic **11** (δ_C) (75 MHz, $CDCl_3$)	^{1}H NMR Data Natural **11** (δ_H) (300 MHz, $CDCl_3$)[42]	^{1}H NMR Data Synthetic **11** (δ_H) (300 MHz, $CDCl_3$)
179.0	179.3	13.20 (broad s, 1H)	11.10 (broad s, 2H)
164.3	163.4	11.10 (broad s, 1H)	
142.3	141.5	7.30 (broad s, 1H)	7.26 (s, 1H)
142.1	141.3	6.63 (d, *J* = 4.2 Hz, 1H)	6.56 (d, *J* = 3.9 Hz, 1H)
125.1	124.4	6.23 (d, *J* = 4.2 Hz, 1H)	6.15 (d, *J* = 3.9 Hz, 1H)
114.3	113.5	5.90 (s, 1H)	5.87 (s, 1H)
113.0	112.1	3.90 (s, 3H)	3.86 (m, 3H)
111.7	110.9	3.33 (m, 2H)	3.28 (m, 2H)
106.0	105.3	2.14 (m, 1H)	2.09 (s, 3H, $CH_3CO_2^-$)
91.5	90.8	2.10 (s, 3H, $CH_3CO_2^-$)	1.73 (m, 1H)
58.9	58.2	1.56 (m, 2H)	1.46 (m, 1H)
57.8	57.0	1.30 (m, 3H)	1.19 (m, 1H)
36.6	35.8	0.98 (t, *J* = 8.0 Hz, 3H)	0.93 (m, 6H)
27.2	26.5		
24.1	24.6		
17.4	16.7		
11.8	11.1		

A.2 APPENDIX TWO

X-ray crystal structure report for compound 53

A full X-ray crystallographic report for compound **53** (as compiled by Anthony C. Willis of the Australian National University) is provided in PDF-format on the compact disc found on the inside back cover of this thesis.

X-ray report 53.pdf

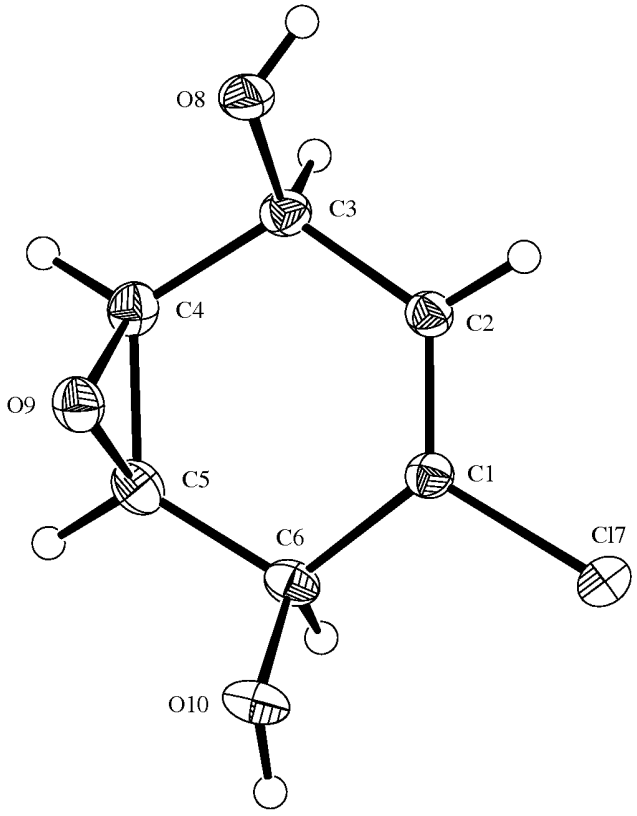


A.3 APPENDIX THREE

X-ray crystal structure report for compound 94

A full X-ray crystallographic report for compound **94** (as compiled by Anthony C. Willis of the Australian National University) is provided in PDF-format on the compact disc found on the inside back cover of this thesis.

X-ray report 94.pdf

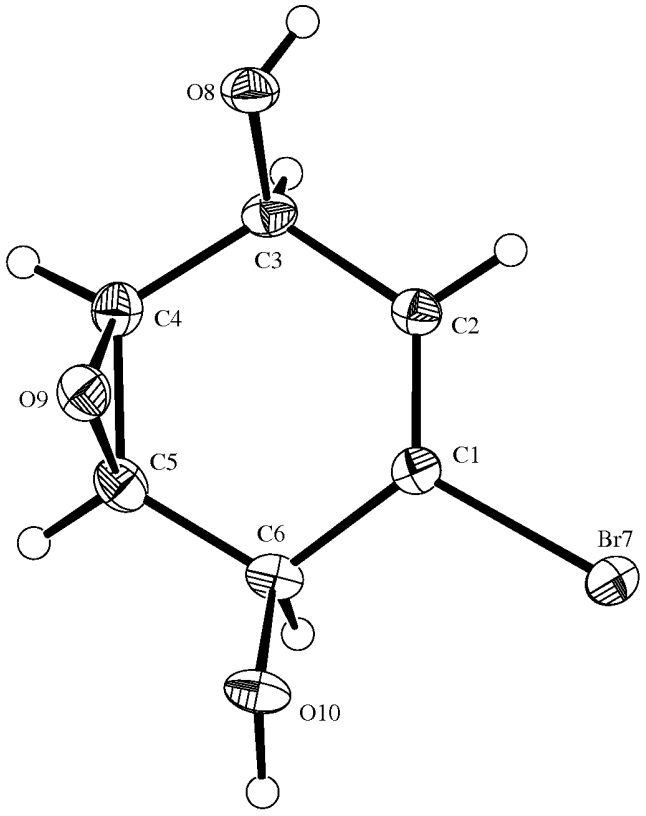


A.4 APPENDIX FOUR

X-ray crystal structure report for compound 95

A full X-ray crystallographic report for compound **95** (as compiled by Anthony C. Willis of the Australian National University) is provided in PDF-format on the compact disc found on the inside back cover of this thesis.

X-ray report 95.pdf

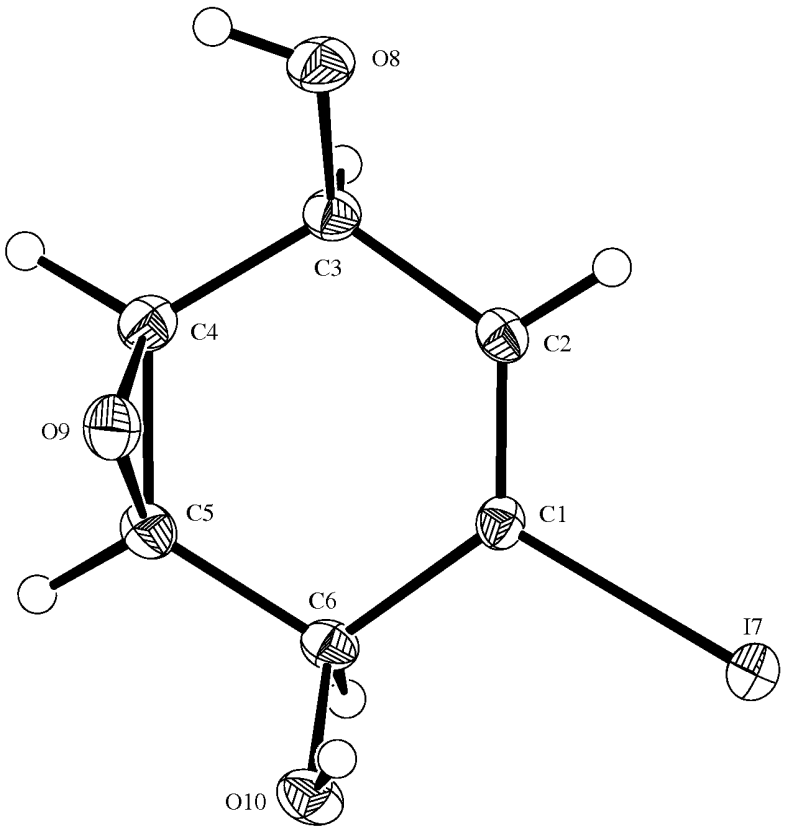


A.5 APPENDIX FIVE

X-ray crystal structure report for compound 96

A full X-ray crystallographic report for compound **96** (as compiled by Anthony C. Willis of the Australian National University) is provided in PDF-format on the compact disc found on the inside back cover of this thesis.

X-ray report 96.pdf

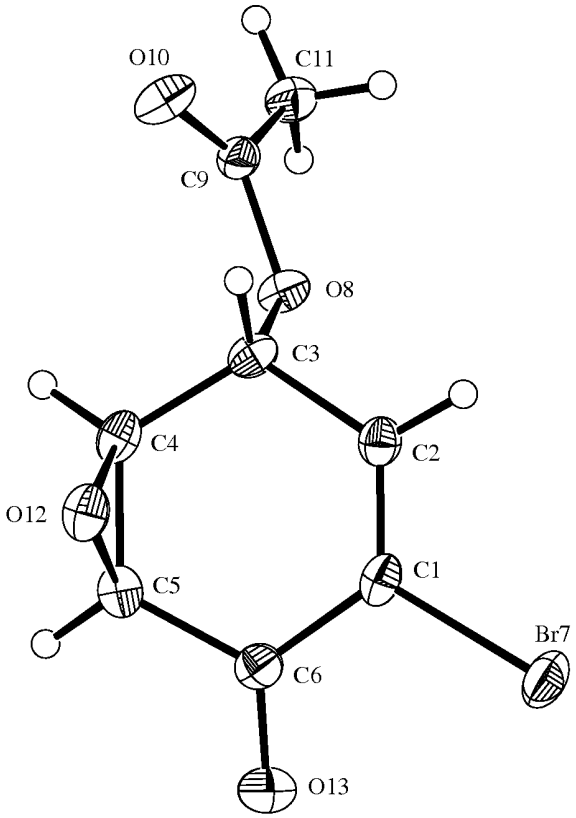


A.6 APPENDIX SIX

X-ray crystal structure report for compound *ent*-106

A full X-ray crystallographic report for compound *ent*-**106** (as compiled by Anthony C. Willis of the Australian National University) is provided in PDF-format on the compact disc found on the inside back cover of this thesis.

X-ray report ent-106.pdf



A.7 APPENDIX SEVEN

X-ray crystal structure report for compound 233

A full X-ray crystallographic report for compound **233** (as compiled by Anthony C. Willis of the Australian National University) is provided in PDF-format on the compact disc found on the inside back cover of this thesis.

X-ray report 233.pdf